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ON TWO HELMINTHS OF DOMESTIC RUMINANTS IN INDIA[1]

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(With 6 Figures in the Text)

Cymbiforma indica, *n.sp.*

Numerous specimens of this fluke were obtained on several occasions from domestic ruminants which were examined at Mukteswar. It has been ascertained that the infection occurs very commonly among goats and sheep, and occasionally also among bulls. Two buffaloes were also examined for infection with these flukes, but with negative results. The intensity of infection, particularly among ovines, is very great, several hundred specimens being obtained every time. Although these worms are found in all parts of the alimentary canal posterior to the oesophagus, the usual seat of predilection is the small intestine. On one occasion only were a few worms found in the bile ducts. It has been observed that the flukes die soon after the death of the host and begin to undergo post-mortem changes. For this reason it was often found difficult to obtain satisfactorily stained preparations of the parasites. Usually acid haematoxylin gave better results than the various carmine stains. The following description of the worms is based on the observation of several unstained and stained preparations and eight series of sections made through various planes.

In the fresh condition the anterior half of the body appears somewhat translucent, and the posterior half, which contains most of the organs, is greyish white. On fixation the entire body appears greyish white. In their natural condition, the worms appear more or less pear-shaped, the posterior extremity being broad and rounded, and the anterior portion of the body gradually tapering to a blunt point. The dorsal surface of the body is slightly curved and the margins are turned ventrally. When viewed ventrally the worms appear more or less boat-shaped. They measure 0·8–2·715 mm. in length, and their maximum breadth, which is attained in the region of the cirrus-sac, is 0·31–0·96 mm. The cuticle of the body is smooth. The longitudinal muscle fibres are well developed and are seen prominently underneath the cuticle throughout the body. Yamaguti (1933) remarks that in *Cymbiforma sikae* the longitudinal musculature forms powerful strands at the free lateral borders of the body. This condition does not obtain in the species now being described. The strands of the longitudinal musculature are developed as powerfully on the dorsal surface of the body as on its free lateral borders. On the ventral surface, however, the longitudinal musculature is comparatively poorly developed. As in *C. sikae* the diagonal fibres lying under the longitudinal muscle layer form a characteristic network on the concave side of the anterior half of the body. Numerous glands are present.

The mouth is situated terminally at the anterior end, and is surrounded by the oral sucker, which is round and measures 0·067–0·14 mm. in diameter. A pharynx is absent. The oesophagus is slender and measures 0·05–0·148 mm. in length. Normally it is cylindrical and of a uniform calibre throughout its length, but occasionally it appears enlarged posteriorly, presumably owing to slight contraction. Unicellular glands surround the oesophagus, and these appear to be more prominent in some cases than in others.

[1] Paper read before the Medical and Veterinary section of the 27th Indian Science Congress, Madras, 1940.

Normally the oesophagus commences at the posterior border of the oral sucker, but in contracted specimens it appears to overlie the latter organ. The intestinal caeca are also slender and at their commencement pass gradually towards the periphery of the body. They run almost parallel to each other for a short distance in front of the vitellaria, but thereafter they gradually converge and finally terminate in the intertesticular area, opposite the middle or the posterior third of the testes.

The transverse nerve band is very prominent and measures 0·009–0·01 mm. in breadth. It lies dorsally to the anterior half of the oesophagus and is situated at a distance of

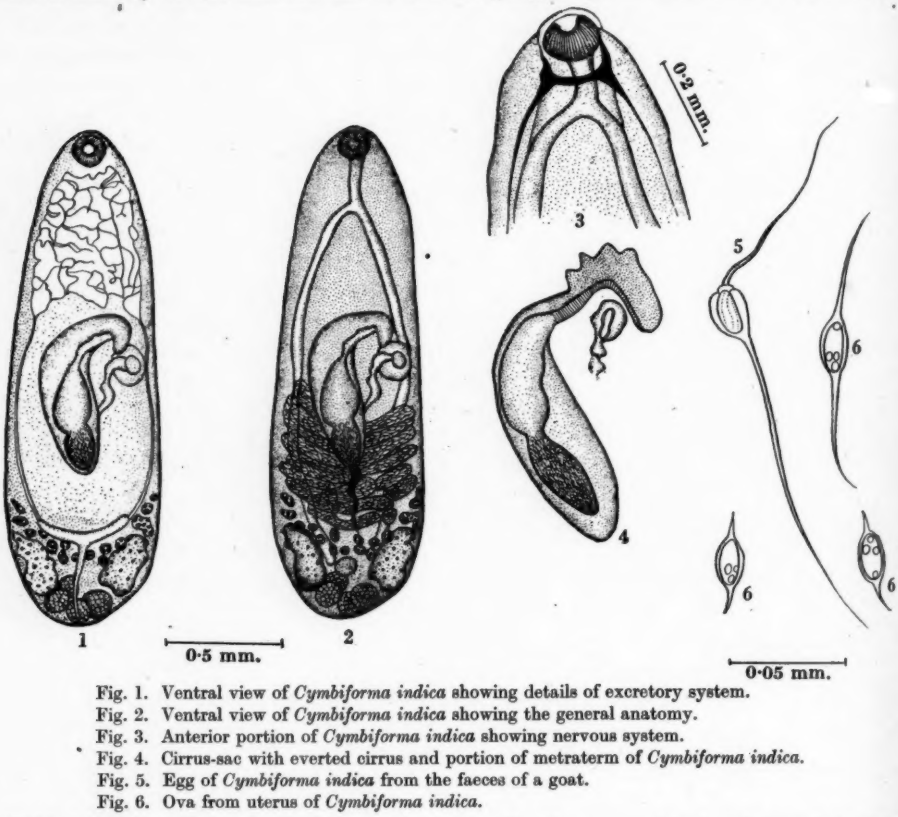

Fig. 1. Ventral view of *Cymbiforma indica* showing details of excretory system.
Fig. 2. Ventral view of *Cymbiforma indica* showing the general anatomy.
Fig. 3. Anterior portion of *Cymbiforma indica* showing nervous system.
Fig. 4. Cirrus-sac with everted cirrus and portion of metraterm of *Cymbiforma indica*.
Fig. 5. Egg of *Cymbiforma indica* from the faeces of a goat.
Fig. 6. Ova from uterus of *Cymbiforma indica*.

0·039–0·073 mm. from its anterior end. Laterally the nerve band enlarges into two triangular ganglia from which nerves pass both anteriorly and posteriorly.

The excretory pore is situated subterminally, slightly towards the dorsal side. It leads into a tubular bladder which bifurcates into two arms at about the level of the anterior third of the testes. The arms diverge laterally almost as far as the anterior extent of the vitellaria, but usually a few follicles on each side may be seen anteriorly to the level of these arms. An excretory duct arises from each of these arms and passes anteriorly along the side of the body. Immediately in front of the level of the cirrus-sac it bifurcates into two branches which pass anteriorly almost as far as the oral sucker. These two branches give off numerous sub-branches which form an intricate network

between the oral sucker and the anterior border of the cirrus-sac. This network is clearly visible in fresh specimens.

The testes are irregularly lobed bodies, lying opposite to each other near the posterior end of the body, and measuring 0·168–0·550 × 0·068–0·26 mm. Sometimes the lobes are quite well defined, having the appearance of a cauliflower, but occasionally the testes are so compact that they appear sausage-shaped, as described by Yamaguti for *C. sikae*. The vasa efferentia arise from the internal face of the testes, at about their centre, pass centrally towards one another and unite to form a vas deferens. This latter passes in front, almost centrally, in a slightly zigzag manner, and enlarges into a convoluted vesicula seminalis externa which enters the cirrus-sac. This sac is the most prominent structure in the body, and measures 0·46–0·75 × 0·125 mm. From the genital atrium it passes first towards the middle of the body and then posteriorly, almost in the middle line. Posteriorly it narrows down almost to a point, but may be bluntly rounded as in *C. sikae*. The position of its posterior extremity with respect to the anterior extension of the vitellaria shows many interesting variations. Sometimes it terminates considerably in front of the level of the anterior extent of the vitellaria, while in other cases its posterior end and the anterior vitelline follicles may lie at the same level. Occasionally, however, vitelline follicles may be seen much in advance of its posterior extremity. The vesicula seminalis interna is situated at the hinder end of the cirrus-sac and measures 0·22–0·28 × 0·075–0·1 mm. The pars prostatica is large, measures 0·275–0·31 × 0·165–0·122 mm., and is constricted from the vesicula seminalis by a small, narrow duct. It is surrounded by numerous unicellular prostate gland cells. The ductus ejaculatorius and the cirrus are both convoluted normally, but they are capable of considerable extension. The cirrus is capable of being extruded from the cirrus-sac, and in such a condition it is seen to be covered with large papillae. The everted cirrus is 0·12 mm. long by 0·03 mm. thick. The opening of the genital atrium lies on the left of the middle line, slightly in front of the midbody. The anterior portion of the body being highly contractile, the genital pore appears to be situated at about the anterior third of the body in specimens which have undergone contraction.

The ovary lies at the extreme posterior end of the body and consists of four lobes which are connected together anteriorly. Each of the four lobes measures 0·077–0·145 × 0·063–0·08 mm. The lobes in the majority of cases are entire, but not infrequently they may be subdivided. In one extreme case it was found that three out of the four ovarian lobes were subdivided into three lobules each. Sometimes only two or three of the four lobes may be seen at one time. The ovarian lobes may touch the testes laterally or they may be separated by a small space. The oviduct passes anteriorly for only a short distance and enlarges to form the oötype. This latter is surrounded by a centrally situated shell-gland which measures 0·1–0·17 × 0·07–0·13 mm. The Laurer's canal arising from the oviduct opens externally on the dorsal side, in the region of the shell-gland. A receptaculum seminis is absent. The vitellaria are composed of large follicles which are arranged in the form of a horse-shoe, semicircle or shallow curve. Some of the follicles lie dorsally to the testes, some are situated laterally and in front of them, while a large number lie in the intertesticular area. Stout vitelline ducts arise immediately anterior to the testes, meeting centrally to form a vitelline reservoir which lies dorsally to the shell-gland, and from which a small duct leads to the ootype. The uterus continues distally into a well-developed metraterm which lies on the left side of the cirrus-sac and measures 0·14–0·37 × 0·06–0·165 mm. It is lined internally with very thick cuticle and is very muscular.

It opens into the genital atrium on the left side of the opening of the cirrus-sac. The eggs are numerous and oval, with filaments at either end, and measure 0·018–0·0375 × 0·011–0·0135 mm. The filaments of the eggs are of varying lengths and may measure 0·008–0·0198 mm. The length of the filaments usually depends on the maturity of the eggs, it having been found that eggs having a smaller number of segments have also smaller filaments than those with a larger number of segments. Even the filaments at the two ends of the same egg are not necessarily equal. The eggs in the uterus are at various stages of development and consequently have a varying number of segments. Those in the faeces of the host are rather longer and wider than the above and contain a developed embryo.

Discussion

From the foregoing description it is quite apparent that the form in question is a monostome belonging to the family Notocotylidae and to the genus *Cymbiforma* Yamaguti, 1933. This genus was created by Yamaguti for the species *C. sikae* from *Sika nippon* in Japan, and up to the present no other species has been assigned to the genus. In general anatomical features the Indian form resembles the Japanese one, but can be readily distinguished from it by the shape and musculature of the body, the course and termination of the intestinal caeca, the anterior extent of the uterine coils, the position of the shell-gland, the number of lobes of the ovary and the dimensions of the eggs. Yamaguti has not observed the Laurer's canal in *Cymbiforma sikae*, but it is present in the Indian form. Yamaguti does not mention the structure known as the vesicula seminalis externa, but possibly the degenerate nature of the material at his disposal did not bring out this structure sufficiently prominently to attract his attention. In a large number of preparations at the writer's disposal this structure was very difficult to see, but in others it could easily be distinguished. It may therefore be presumed that this structure is present also in *C. sikae* although Yamaguti failed to notice it. A similar presumption may also be made regarding the Laurer's canal. For the reasons stated previously the Indian form is regarded as a new species, for which the name *C. indica* is proposed.

In view of the anatomical features presented by the Indian form it has been considered necessary to modify the definitions of the genus *Cymbiforma* and the subfamily Cymbiforminae originally proposed by Yamaguti (1933).

Emended diagnosis of Cymbiforminae

Notocotylidae: Lateral margins of body inflexed to form a ventral groove. Integument unarmed. Ventral glands absent. Intestinal caeca terminating in front of posterior extremity of body. Genital pore submedian, pre-equatorial. Testes elongated, lobed, close to lateral body-wall. Cirrus-sac large, approximately equatorial, partially enclosing vesicula seminalis. Ovary at posterior extremity of body. Vitellaria follicular, dorsal to testes. Uterus not extending anterior to cirrus-sac, partly extracaecal. Excretory bladder with a fairly long stem and fairly short arms. Parasitic in the alimentary canal of mammals.

Type genus. *Cymbiforma* Yamaguti, 1933.

Emended diagnosis of Cymbiforma

Cymbiforminae: Body approximately canoe-shaped. Diagonal fibres of subcuticular musculature forming characteristic network on ventral side of anterior half of body.

Oral sucker terminal. Testes deeply or slightly lobed. Cirrus-sac very conspicuous, lying across body and enclosing vesicula seminalis interna, pars prostatica with numerous glands, ductus ejaculatorius and a long, convoluted and eversible cirrus. Genital atrium present. Ovary lobed. Uterus disposed in transverse coils. Metraterm wide, well developed, opening on left side of male aperture. Eggs with polar filaments of varying length.

Type species. *C. sikae* Yamaguti, 1933.

Specific diagnosis of Cymbiforma indica *n.sp.*

Cymbiforma: Length 0·8–2·715 mm. Maximum breadth 0·31–0·96 mm. Longitudinal subcuticular muscles developed equally on the dorsal side and the free lateral portion of body. Oral sucker 0·067–0·14 mm. in diameter. Oesophagus 0·05–0·148 mm. long, surrounded by unicellular glands. Intestinal caeca terminating at the middle of the testes or at their posterior third. Testes measure 0·168–0·560 × 0·068–0·26 mm. Cirrus-sac measures 0·46–0·75 × 0·125 mm. Vitellaria extending anterior to testes. Ovary consists of four lobes, each 0·077–0·145 × 0·063–0·08 mm. Laurer's canal present. The uterine coils extend from the shell-gland to about the middle of the cirrus-sac. Eggs measure 0·018–0·0375 × 0·011–0·0135 mm. Polar filaments 0·008–0·0198 mm. long.

Hosts. Goats, sheep and cattle.

Location. The whole of the alimentary canal following oesophagus, bile ducts.

Locality. Mukteswar-Kumaun, U.P., India.

Type and co-type material deposited in the helminthological collection of the Imperial Veterinary Research Institute, Mukteswar-Kumaun, U.P., India.

Capillaria bilobata *Bhalerao*, 1933

These worms were obtained by the writer in 1933 from the abomasum of cattle at Mukteswar. Recently a few worms were also collected from the small intestine of a goat in this locality, and these on examination proved identical with the specimens from cattle. As remarked previously (Bhalerao, 1933), these worms are very peculiar in that the males have a bilobed spicular sheath, a characteristic without parallel among the species assigned to the genus *Capillaria*. Measurements of the important organs of the two largest specimens in the collection are as follows:

Male. Length 16·85 mm., maximum thickness 0·057 mm., oesophagus 8·68 mm. long, spicule 0·195 mm. long, spicular sheath 0·062 mm. long, and lateral alae 0·087 mm. long.

Female. Length 19·42 mm., maximum breadth 0·074 mm., oesophagus 8·32 mm. long, vulva 8·47 mm. from anterior end, mature eggs measure 0·047–0·052 × 0·018–0·02 mm.

The writer wishes to record here his appreciation of the assistance rendered by Mr Karam Chand, of the Punjab Veterinary Service, in the collection of material and in the preparation of some valuable slides.

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ON A NEW SPECIES OF TREMATODE FROM A SPOON-BILL IN AFGHANISTAN

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(With 1 Figure in the Text)

Through the courtesy of Prof. Ali Akhtar, Afghanistan, the writer examined about eight flukes obtained from the trachea of the spoon-bill, *Platalea leucorodia* var. *major*. A short account is given of the species, and its affinities are discussed below.

The worms are large, elongated, flat and differentiated into small anterior and large posterior portions. There is a very faint indication of the shoulders separating the anterior portion of the body from the posterior. The former portion is almost triangular, with a rounded extremity. The latter portion of the body tapers gradually as far as the posterior extent of the testes. A small part of the body containing only a portion of the excretory bladder is constricted off from the rest of the body, and thus appears as a small caudal appendage. The worms are not very thick, and consequently the gross features of their anatomy can be made out with the naked eye. The margin of the body is slightly undulating. The cuticle is thin and devoid of any armature. Below the cuticle there is a layer of circular muscles and below this there are longitudinal muscles. Below the longitudinal muscles are transverse muscles disposed in such a manner as to give the impression of bamboo matting. A special development of the longitudinal muscles acting as retractors is seen in connexion with the oral sucker. The worms measure 22–30 mm. in length, and their maximum breadth, which is attained in or about the region of the ventral sucker, is 3·2–3·47 mm. The mouth is subterminal and is surrounded by an oral sucker measuring 0·87–1·03 × 0·88–1·07 mm. The ventral sucker is larger than the oral, measuring 1·2–1·68 mm. in diameter, and is situated at a distance of 2–2·3 mm. from the anterior end of the body. The oral sucker is followed by the pharynx, which is oval and measures 0·47–0·56 × 0·36–0·41 mm. The oesophagus is very small, and there is usually a tendency for it and the intestinal fork to lie dorsally to the pharynx. The intestinal caeca have a slightly irregular outline. They pass along the sides of the body, almost midway between the vitellaria externally and testes internally, and terminate slightly in front of the posterior constriction of the body. Occasionally one caecum is observed to terminate slightly anteriorly to the other.

The excretory pore opens centrally at the posterior end of the body. It leads by a very short canal into a very long and tubular excretory canal which bifurcates into two arms slightly behind the ovary.

The genital pore is situated centrally, immediately behind the pharynx, occasionally being partially overlapped by the latter. The testes are extremely numerous. In one case 380 were counted and in another 391. They extend centrally into the intercaecal area, from slightly behind the ovary to a level 0·88–1·1 mm. from the posterior end. They are of various shapes and sizes and measure 0·14–0·47 × 0·13–0·24 mm. As observed by Nicoll (1914) for *Orchipedum sufflavum*, the testes are disposed in two layers anteriorly, but posteriorly they are in a single layer. The outermost testes partially

overlap the intestinal caeca. Arising from the testes are numerous vasa efferentia which, compared with the size of the testes, are fairly stout. The vasa efferentia unite to form a vas deferens which enlarges into a long, coiled vesicula seminalis. The proximal end of the vesicula seminalis lies medially to the ovary and its coils extend anteriorly, passing dorsally to the ventral sucker on the right side and terminating slightly in front of it. The pars prostatica, surrounded by numerous prostatic cells, appears to be a transversely oval body lying between the pharynx and the ventral sucker, somewhat nearer to the latter. The ductus ejaculatorius is a thin and fairly long duct which opens into the genital atrium. There is no cirrus-sac.

Fig. 1. Dorsal view of *Orchipedum akhtari* n.sp.

The ovary, measuring 0·58–0·70 × 0·46–0·55 mm., is an oval body. As a rule it lies postero-laterally to the ventral sucker on the right side, but occasionally it may be situated in a similar position on the left side. It is separated from the ventral sucker by the uterine coils. The receptaculum seminis is semilunar, lies immediately behind the ovary, and measures 0·43–0·66 × 0·14–0·21 mm. The Laurer's canal is wide, muscular, somewhat convoluted, and arises directly from the receptaculum seminis. The vitellaria are very voluminous and extend laterally, in broad bands, from the level of the pharynx to a small distance from the posterior end. The glands of either side do not unite with each other posteriorly. A large majority of the vitelline follicles are extracaecal, but a few internal ones overlap the intestinal caeca both dorsally and ventrally. In addition to these large extracaecal bands of vitelline follicles there are two intercaecal bands. These lie medially to the intestinal caeca and in places are discontinuous. They vary very considerably in extent. The longitudinal vitelline ducts pass internally to the outer vitelline bands and unite to form transverse vitelline ducts which pass close behind the ovary. The uterine coils fill the space between the ovary and the intestinal caecum of the opposite side, pass dorsally to the ventral sucker, and extend anteriorly as far as the pars prostatica. The terminal portion of the uterus, the metraterm, though wider than the ductus ejaculatorius, is slightly smaller. The eggs measure 0·071–0·078 × 0·038–0·043 mm.

From the above description it is apparent that the form in question belongs to the genus *Orchipedum*, assigned to the family Orchipedidae. The genus *Orchipedum* was created by Braun (1901). Braun in 1902 included in this genus the species *Distomum formosum* (Sonsino, 1890). Odhner (1913) excluded *Orchipedum* from his family Psilostomidae. Skrjabin (1913), unaware of Odhner's work, divided the Psilostomidae into two subfamilies, Psilostominae and Orchipedinae. Skrjabin (1924), however, removed the Orchipedinae from the Psilostomidae and gave them the rank of a family, Orchipedidae.

Six[1] species have been so far assigned to the genus *Orchipedum*, viz. *O. tracheicola* Braun, 1901; *O. formosum* (Sonsino, 1890); *O. turkestanicum* Skrjabin, 1913; *O. sufflavum* Nicoll, 1914; *O. armeniacum* Skrjabin, 1915; and *O. centorchis* Witenberg, 1922. Among these species the form described here has a greater affinity to *O. sufflavum* than to any other species of the genus. It can, however, be readily differentiated from this species by the position of the genital pore, the posterior extent of the vesicula seminalis, the distance between the testes and the ventral sucker, the fact that the ovary is longitudinally instead of transversely oval, and by the length of the ductus ejaculatorius. The differences justify the erection of a new species, for which the name *O. akhtari* is proposed in honour of Prof. Ali Akhtar.

Specific diagnosis of Orchipedum akhtari *n.sp.*

Length 22–30 mm., maximum breadth 3·2–3·47 mm. Pharynx 0·47–0·56 × 0·36–0·41 mm. Intestinal fork usually lies dorsally to pharynx. Genital pore immediately behind pharynx. Testes about 400. Vesicula seminalis extending posteriorly as far as middle of ovary. Ductus ejaculatorius fairly long. Ovary longitudinally oval, 0·58–0·70 × 0·46–0·55 mm. Receptaculum seminis semilunar, 0·43–0·66 × 0·14–0·21 mm. Vitellaria extending anteriorly as far as level of pharynx. Eggs 0·071–0·078 × 0·038–0·043 mm.

Host. *Platalea leucorodia* var. *major*.
Location. Trachea.
Locality. Kabul, Afghanistan.

The type slide is deposited in the helminthological collection of the Imperial Veterinary Research Institute, Mukteswar-Kumaun, U.P., India.

The writer wishes to express his indebtedness to Prof. Ali Akhtar for his valuable gift of material upon which this paper is based.

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[1] A seventh species, *O. isostoma* [*isostomum*] (Rud., 1819) is added by Dollfus, Callot & Desportes, 1935 (*Ann. Parasitol. hum. et comp.* **13**, 116–132).—Ed.

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ON THE BIONOMICS OF *APHIDIUS MATRICARIAE* HAL., A BRACONID PARASITE OF *MYZUS PERSICAE* SULZ.

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(With 6 Figures in the Text)

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1. Introduction and methods

This study has arisen from attempts to observe the influence exerted by a parasite on *Myzus persicae* Sulz., the most efficient vector of the potato viruses, leaf roll and '*Y*'. *Aphidius matricariae* Hal. is an internal parasite of *Myzus persicae* Sulz.; it can readily be found in the field and in glasshouses where it can parasitize *M. circumflexus* Buckt. and *Aulacorthum solani* Kalt. (*Myzus pseudosolani* Theob.). The observations have been made mainly from the point of view of the effect of the parasite on the host. It was also found necessary to make certain detailed observations on the early stages of the parasite itself.

The entire colony of the parasite was raised from a single fertilized female which was later identified by Mr J. F. Perkins, of the British Museum, as *Aphidius matricariae* Hal. The parasite was bred on its host *Myzus persicae* Sulz. under glass chimneys.

Sectioning was done with a freezing microtome, and the material was either studied in situ or dissected, mounted and stained in the usual manner. Staining in haematoxylin and counter-staining in alcoholic eosin 1% was found to give good results. Gentian violet stain (1% in 70% alcohol) was also used.

The respiratory system was studied by dissecting out the larva when the host was in moribund stage and immediately mounting in Berlese fluid.

2. The egg

The egg is elliptical, white, and is ornamented with spiral ridges and longitudinal furrows. The length of fourteen eggs varied from 0·05 to 0·07 mm., the breadth from 0·013 to 0·023 mm.

The rate of development depends very largely on temperature. At about 70° F. the eggs take 3–4 days, and at 55° F. 7–9 days to hatch. The egg is usually deposited in the abdominal tissue of the host, where it adopts a rounded shape with a loosely fitting chorion. At 55° F., 48 hr. after oviposition, the embryo begins to part and the future larva does not take on a definite shape until the 5th day. It hatches on the 8th or 9th day. Hatching appears to be the result of internal pressure exerted on the chorion as a result of the active movements of the developing larva.

3. The larva

Being an internal parasite, the various exuviae could not be traced, hence development was noted in terms of stages which were distinguished by a general change of shape and size and alterations in the form of internal organs. Daily dissections showed that at 55° F. each stage lasts for 3 days. The larva was fully grown at what appeared to be the 5th stage.

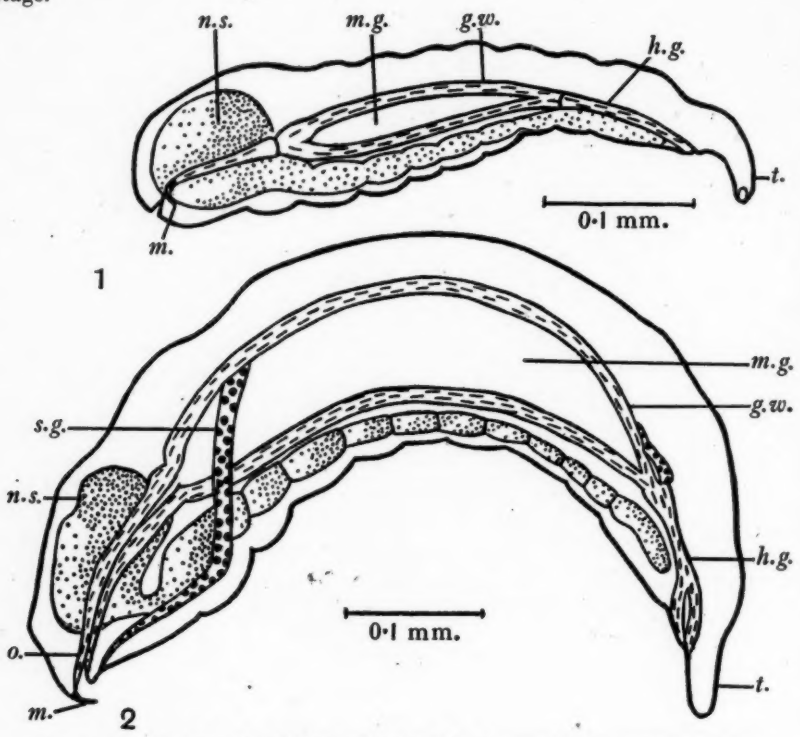

Fig. 1. 1st stage larva of *Aphidius matricariae* Hal. *m.* mandible; *n.s.* nervous system; *m.g.* mid-gut; *g.w.* gut wall; *h.g.* hind-gut; *t.* tail.

Fig. 2. 2nd stage larva of *Aphidius matricariae* Hal. *o.* oesophagus; *s.g.* salivary gland. Other lettering as for Fig. 1.

(*a*) *Morphology.* The larva in the 1st stage (Fig. 1) is almost straight with its head as the largest of 13 segments. The body tapers posteriorly and terminates in a tail-like appendage which disappears in the 3rd stage (Fig. 3). In the 2nd stage (Fig. 2) the larva is curved and tapers at both ends, the head still being the largest of the segments. In the 3rd, 4th and 5th stages the body curvature increases. The ends are more rounded and the body segments are smoother.

In the 1st and 2nd stages the *digestive system* is represented only by the *oesophagus* (*o.*), the *mid-gut* (*m.g.*) and the *hind-gut* (*h.g.*). The *oesophagus* runs through the first two segments. The *mid-gut* extends from the 3rd to the 10th segment; it is a blind tube in the 1st and 2nd stages. In the 3rd stage it is open at both ends and appears to function.

The *hind-gut* is not differentiated into *ileum* (*i.*) and *rectum* (*r.*) until the 4th stage (Fig. 4).

The *salivary glands* (*s.g.*) are first apparent in the 2nd stage. They are inconspicuous, straight tubes overlying the mid-gut. In the fully grown larva the glands are very tortuous and prominent. They extend as far as the 10th segment and fuse into a common duct in the region of the maxilla and labium (Fig. 5).

The *respiratory system* (Fig. 6) is most easily observed when the host becomes moribund and the larva is fully grown. It is represented by well-developed stigmatic branches (*s.b.*) leading into longitudinal tracheal trunks (*l.t.*). Anterior to the first spiracle (*sp.*) the tracheal trunks divide into a number of branches which appear to supply the brain. The anterior loop (*a.l.*) is distinctly arched and connects the two longitudinal tracheal trunks. The imaginal disks of the legs (*i.d.l.*) and wings (*i.d.w.*) are well developed in the fully grown larva (Fig. 5).

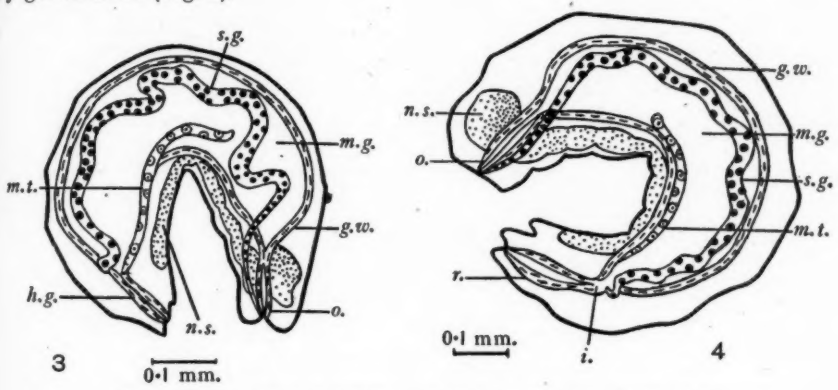

Fig. 3. 3rd stage larva of *Aphidius matricariae* Hal. *m.t.* Malpighian tube. Other lettering as for Figs. 1 and 2.

Fig. 4. 4th stage larva of *Aphidius matricariae* Hal. *r.* rectum; *i.* ilium. Other lettering as for Fig. 3

Table 1. *Showing the number and stages of developing larvae in superparasitized* Myzus persicae

Aphis no.	No. of larvae	Stages of development
1	3	1 in 3rd stage, 2 in 1st stage
2	2	1 in 3rd stage, 1 in 1st stage
3	2	1 in 3rd stage, 1 in 1st stage
4	2	1 in 4th stage, 1 in 1st stage
5–14	1 in each case	1 in 4th stage in each case

(*b*) *Supernumerary larvae.* When *M. persicae* is superparasitized, it is seen on dissection that 6–10 eggs are capable of hatching. For a time the rate of development of larvae from these is the same, irrespective of the number present. Later dissections of aphides superparasitized on the same day showed that only one larva reached the 3rd stage, whilst others always showed signs of degeneration or under-development. The results of the later dissections are shown in Table 1.

In ten cases (in Table 1) only a 4th stage larva was present showing the ultimate disappearance of supernumerary larvae. Timberlake (1910), working on *Praon* sp., studied the question of supernumerary larvae and their final disappearance. He does not suppose

it due to violence or starvation. Spencer (1926) concludes that the inhibition is a biochemical rather than a mechanical process as evidence of mechanical injury has never been seen. Wheeler (1923) suggests four possibilities for the inhibition of supernumerary

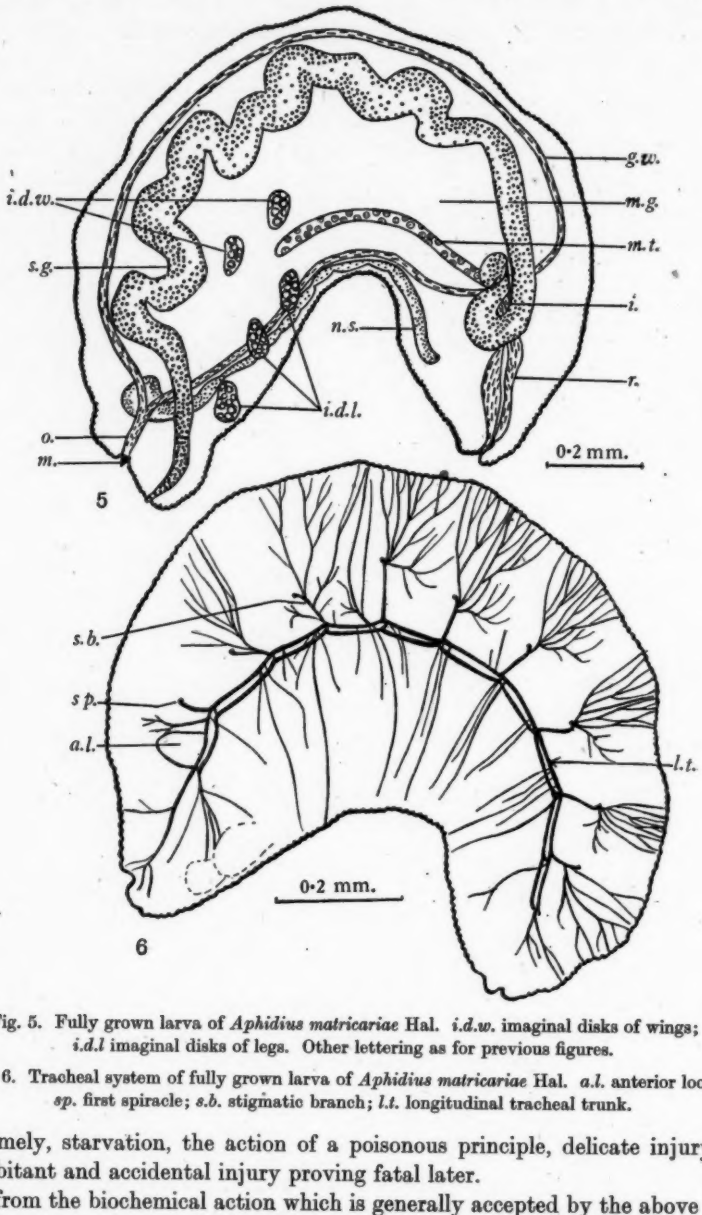

Fig. 5. Fully grown larva of *Aphidius matricariae* Hal. *i.d.w.* imaginal disks of wings; *i.d.l* imaginal disks of legs. Other lettering as for previous figures.

Fig. 6. Tracheal system of fully grown larva of *Aphidius matricariae* Hal. *a.l.* anterior loop; *sp.* first spiracle; *s.b.* stigmatic branch; *l.t.* longitudinal tracheal trunk.

larvae, namely, starvation, the action of a poisonous principle, delicate injury by the older inhabitant and accidental injury proving fatal later.

Apart from the biochemical action which is generally accepted by the above workers, there is only slight evidence of an offensive nature being adopted by the surviving larva.

The following observations, however, suggest that, in the case of *Aphidius matricariae*, one larva at times attacks and destroys the other larva or larvae present. Two cases were observed where a larva had its mandibles imbedded in another larva. In addition, the presence on a number of occasions of mandibles in the mid-gut of a surviving larva strongly suggests that cannibalism occurs more frequently. It is possible that a larva may ingest its own moulted mandibles, but this is unlikely at least until the 4th stage is reached, as there is up to this stage always plenty of food present. Examinations of mid-guts of larvae (from superparasitized *Myzus persicae*) of various stages showed that: (*a*) In each of three fully grown larvae there was a pair of 1st stage mandibles. (*b*) In each of two 4th stage larvae there was in one a pair of 1st stage mandibles and in the other a pair of 2nd stage mandibles. (*c*) In one 3rd stage larva there was a pair of 1st stage mandibles. (*d*) In each of fifteen 2nd stage and twenty-one 1st stage larvae, no mandibles were found in their guts. Until the 2nd stage the mid-gut is a blind tube so that it is impossible for the mandibles to be found within it. Thorpe[1] states that 'the very fact that the first two stages have strongly sclerotised mandibles suggests predatory behaviour'.

(*c*) *Larval habits.* Irrespective of where the egg is deposited, the parasite larva is always to be found in the abdomen of the host where there is abundance of nutritive material. Since this is so (according to Thompson & Parker, 1930) Seurat considered

Table 2. *Duration of parasitic life from the sessile stage to the emergence of imago under different conditions*

Series	No. of sessiles observed	Relative humidity		Temp. °F.		Sessile life in days
		Max.	Min.	Max.	Min.	
Incubator	11	72	47	83	80	4–6
Glasshouse	13	94	67	76	54	6–8
Laboratory	11	71	58	70	66	5–8
Outdoor	93	100	70	61	31	26–34

that one of the functions of the tail (Figs. 1, 2) was locomotion, whilst Timberlake (1910) and Tothill (1922) believe it to be a respiratory organ. Thorpe (1932) later showed it to be of no importance in respiration. Thompson & Parker (1930) doubt whether this organ is physiologically of any importance. It is possible that the tail helps the young larvae of 1st and 2nd stages to move about for predatory purposes. In the 3rd stage the tail has disappeared and the larva feeds voraciously on the host tissues and on any supernumerary larvae. The mid-gut is now open and solid bodies including mandibles are present inside the gut. Reproduction of the host ceases but the larva avoids touching the vital systems. It is only in the fully grown stage that the larva attacks the digestive system, for prior to this it would mean its own premature starvation. Before pupation the larva secures the dead host to any object within its reach.

(*d*) *Larval and sessile periods.* The number of days which elapsed between the oviposition of the egg and the host becoming sessile have been noted. Under heated glasshouse conditions (with temperatures ranging from 40 to 52° F.) in winter months, the time between oviposition and the moribund stage of the host varied from 15 to 28 days. The fully grown larva ceaselessly makes preparations for pupation, and at the same time the host becomes sessile. This sessile period varies from 5 to 33 days under heated glasshouse conditions, the variation being largely due to temperature. The sessile period under varying conditions is shown in Table 2.

[1] Remarks in a letter.

The act of pupation and its duration were studied by regular dissections. For this purpose a humid condition was essential, otherwise the pupa desiccated very quickly. Within 6 hr. of the death of the host a longitudinal furrow appears on the abdominal venter. This furrow is torn to form a slit, through which the larva throws out strands of silk so as to attach the host to any nearby object. After frequent orientations within the host, during which the walls are plastered with silk, the larva pupates.

4. The pupa

When the larva has completed plastering the walls of the sessile host with silk strands, it becomes less restive and begins to defaecate flaky excreta. From the outside this is a rough indication of pupation.

Twenty-four hours after the opening of the slit in the abdominal venter of the host, the imaginal eyespots (underneath the larval skin) begin to appear. In the majority of cases the pupa emerges from the larval skin after 5 days. The fresh pupa is creamy yellow with bright red eyes. Forty-eight hours later the thorax and abdominal dorsum begin to darken and these become almost black after a further lapse of 24 hr. This is the pre-imago stage. After a day the parasite imago begins to unfold its wings and legs, the former becoming fully expanded within 10–15 min. These changes were observed in twenty-two cases at 65–67° F.

The sex of a pupa can be ascertained by means of the antennal sheath, which reaches the 3rd abdominal segment in the male and is shorter in the female. The abdomen in the male is short and thickset, whilst in the female it is long, narrow and tapers to a point.

5. The Imago

The emergence of the adult is generally effected by cutting out a circular lid in the abdominal dorsum of the sessile host. The act of emergence was observed in 100 cases—in ten cases the lid was made in the thorax of the sessile host and in ninety cases in the abdomen, generally between the cornicles. In cases when the host became sessile on the same day there was a tendency for the males to emerge first. Over 80% emerged during night time. Out of 3483 apterous sessiles and 106 alate sessiles, 79·84 and 90·60% respectively of the parasites emerged successfully.

(*a*) *Mating.* Unlike some parasites in which copulation took place soon after emergence, this parasite required some time to elapse between emergence and mating. The earliest mating observed took place 2 hr. after emergence. This sexual instinct is lost earlier in the female than in the male. The total number of matings observed was 116, in twenty-one cases either the male or the female was more than 7 days old (viz. 7–13 days). Many unsuccessful attempts were made to mate females which had already commenced ovipositing parthenogenetically. Out of 126 females handled, ten refused to mate during the whole of their lifetime, in spite of bringing them together with different males which were later proved to be sexually potent. One male may mate with a number of females. Three separate males fertilized thirteen, eighteen and thirteen females respectively. The progeny of the last two of these males (named A and B) is shown in Table 3.

It will be noticed that in early matings the female offspring preponderated, but in the later matings the sex ratio of the progeny was approximately equal.

Usually a male approaches a female fluttering its wings, then mounting, it grips the female and rocks it sideways with occasional movements of the antennae. The female is

much less active and remains almost motionless. This courting period varies from 7 to 135 sec. The copulation period ranges from 42 to 135 sec. It is shortened with increasing temperatures. This species is arrhenotokous, and males produced parthenogenetically were found to mate and produce normal offspring.

(*b*) *Oviposition.* Generally at least 2 hr. elapsed between emergence and oviposition. In the case of fertilized females, oviposition took place within 2–70 min. of mating. A female which had not mated due to absence of a male commenced oviposition no sooner than 2 hr. after emergence. A female, when ovipositing, bends forward its abdomen whilst balancing on its legs. The actual process of oviposition takes only a fraction of a second.

The female parasite in the excitement of oviposition remains very active for the first 2 or 3 days. It stabs in sessile or the moribund hosts, in the legs, antennae, cornicles, cauda or bits of paper—in short, on anything it comes across. Each strike does not mean that an egg is laid and the host successfully parasitized. At times two eggs were

Table 3. *Progeny of successive matings of two male* Aphidius matricariae

Female no.	Progeny of the male parasite A		Progeny of the male parasite B	
	Males	Females	Males	Females
1	63	98	—	—
2	32	140	15	42
3	7	34	—	—
4	8	44	26	38
5	17	46	53	83
6	8	16	44	45
7	17	27	47	50
8	51	93	84	72
9	21	14	11	12
10	16	30	16	10
11	—	—	59	64
12	16	23	25	26
13	13	28	129	128
14	67	52		
15	76	82		
16	73	73		
17	18	23		
18	16	1		

deposited in one single strike, but this occurred only when the female parasite had no access to the host for nearly a week after emergence.

When only one strike was allowed per aphis, 340 out of 1463 aphides became parasitized (i.e. 23·2%); when two strikes were allowed per aphis, forty-four out of the ninety-six aphides struck were parasitized (i.e. 45·7%); where three strikes were allowed, forty out of the fifty aphides struck were parasitized (i.e. 80%), and when four strikes were allowed all sixteen aphides struck were parasitized.

The number of eggs produced by a single female varied from 180 to 350, but the number of strikes made may be greater. Single females were allowed to oviposit for an hour per day ($\frac{1}{2}$ hr. in the morning and in the evening). In the case of five females which on an average made 192 single strikes in their lifetime, 27·5% of the aphides became parasitized. It may be seen from Table 4 that the activity in oviposition by the five females reached its climax on the 2nd or 3rd day of oviposition.

Table 5 shows the number of sessiles obtained, when single mated females were liberated with an unlimited number of aphides. Sessiles were collected daily, and the

number obtained at 3-day intervals is recorded in the table. The sex of the parasites which subsequently emerged is also shown. It will be seen that the number of sessiles collected gradually diminished and very few resulted after the fifth collection (i.e. after the 15th day); also that the proportion of males increases gradually as oviposition advances.

The results shown in Table 5 are in agreement with those shown in Table 4, where the greatest activity occurred in the 2nd or 3rd day of oviposition.

Usually a parasite prefers to stand to the side or behind the host while ovipositing. There were, however, occasions when an excited female, by approaching its host from the front, scared one host after another till practically the whole of the colony was

Table 4. *Number of strikes made on successive days*

Female no.	Strikes on successive days										Total single strikes	No. of sessiles
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th		
1	34	59	46	31	Dead	—	—	—	—	—	170	51
2	79	102	72	21	33	7	5	3	1	Dead	323	46
3	17	—	31	29	14	—	—	14	8	Dead	113	18
4	9	28	15	43	Dead	—	—	—	—	—	95	25
5	30	43	50	34	19	24	26	19	14	Dead	259	108

Table 5. *Record of sessiles and progeny obtained from single mated females*

M. = males, F. = females.

Female no.	No. of sessiles obtained at 3-day intervals													
	1st		2nd		3rd		4th		5th		6th		7th	
	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
1	8	33	10	41	18	16	19	5	8	3	—	—	—	—
2	8	55	5	22	9	25	2	19	5	18	3	1	—	—
3	4	18	1	12	2	3	0	1	—	—	—	—	—	—
4	3	16	1	12	4	15	—	—	—	—	—	—	—	—
5	4	12	6	7	5	6	1	4	—	—	—	—	—	—
6	15	38	19	26	13	13	12	3	—	—	—	—	—	—
7	7	7	11	10	1	6	2	0	—	—	—	—	—	—
8	4	17	10	10	2	3	—	—	—	—	—	—	—	—
9	8	8	8	12	2	3	—	—	—	—	—	—	—	—
10	7	12	6	22	8	15	8	16	17	7	—	—	—	—
Total of both sexes	284		251		169		92		58		4		0	
Ratio of male : female	1 : 3·17		1 : 2·26		1 : 1·64		1 : 1·09		1 : 0·93		1 : 0·33		—	

disturbed and began to disperse. Out of 852 strikes observed, 14·9% were made from the front, 30·7% from behind, and 54·4% from the side of the host. The favourite site for oviposition is that part of the abdomen in the region of the cornicles. The parasite usually avoids ovipositing in the posterior abdominal segments, possibly because there is risk of its making contact with the 'honey-dew' of the host. Once the parasite makes contact with this fluid it often gets exhausted in getting rid of it, and if the ovipositor is smeared further egg laying is improbable.

When the eggs are laid in the cornicles, legs and antennae, they fail to hatch; in a few cases when the egg was deposited in the cauda, parasitization was successful. Out of forty-five strikes observed, twenty were made in the abdominal segments anterior to the cornicles, ten were made between the cornicles and the cauda, eight in the head and thorax, six in the legs and one in the cornicles.

The parasite appeared to be more active when ovipositing in bright light. In order to measure the intensity of light and to correlate it with the activity in oviposition, strips of daylight paper, 'Seltonia', were exposed during the egg laying. The time allowed for oviposition was 30 min., both in the morning and evening. No correlation was found between the light intensity and the number of strikes.

6. Factors other than meteorological factors affecting the colonization of the parasite

(*a*) *Progeny of the parasite.* The total progeny of sixty females was 5603 imagines; the lowest number obtained from a single female was thirteen and the highest was 309. To test the prolific nature of the parasite, seven females (mated and later proved fertilized) were individually liberated under a glass chimney, with twenty-five adult apterous *Myzus persicae* breeding on tulip. One chimney (namely, no. 4) had aphides only to act as a control. The life cycle of the parasite under the existing conditions was completed in 2½–3 weeks' time. The results are shown in Table 6.

Table 6. *Number of sessiles and apparently non-parasitized* Myzus persicae *after liberating individual parasites*

		No. of sessiles and non-parasitized aphides			
Chimney no.	After period of	Sessiles obtained	Apparently non-parasitized aphides	After period of	Apparently non-parasitized aphides
1	5 weeks	115	12	11 weeks	0
2	5 ,,	68	27	11 ,,	0
3	5 ,,	123	18	11 ,,	0
(Control) 4	5 ,,	—	More than 350	11 ,,	Countless
5	4 ,,	57	8	11 ,,	55
6	4 ,,	86	16	11 ,,	10
7	4 ,,	65	19	11 ,,	15
8	4 ,,	49	20	11 ,,	0

It appears that within 11–12 weeks' time (roughly in four generations of the parasite), a single parasite (under confinement in a glass chimney) is able to eradicate an aphis colony originally numbering twenty-five *M. persicae.*

(*b*) *Sex ratio.* The sex of the progeny of unmated females is exclusively male. When parasites are liberated in a glass chimney for breeding purposes, the sex ratio of male: female :: 1 : 1·4, whilst that of an observed mated female was 1 : 2·4. The reason for the narrower ratio in the former case is that, if females are not mated after 2 hr. of emergence they begin ovipositing, and once this occurs mating is improbable.

It will be seen from Table 5 that although the mated female does reproduce both the sexes, the sex ratio tends to become narrower towards the end of oviposition; more males are also produced from later successive matings (vide Table 3).

(*c*) *Longevity of the parasite.* At lower temperatures, such as those prevailing in winter months, the imago lives twice as long, but is not so active and fewer sessiles are obtained. It is strongly heliotropic and its life is lengthened in diffused light. At temperatures of about 42° F. the female parasites lived for 3 weeks under glass chimneys out of doors.

(*d*) *Dissemination of the parasite.* A small number of *Aphidius matricariae* were caught in the mechanical insect trap at Aber, Carnarvonshire (vide Thomas & Vevai, 1940). The parasite appears to fly very readily under normal field conditions. Its flight seems

to be favoured by the factors that govern the flight of aphides in general, namely, wind velocity under 5 miles per hour, temperature above 70° F., and relative humidity below 70°; these parasites were practically all caught when alate aphides were trapped in large numbers. Under laboratory conditions the parasite becomes sluggish at temperatures nearing freezing-point and was observed ovipositing only on three occasions at temperatures below 40° F.

The parasite may also be carried about by its host. Oviposited apterous *Myzus persicae* which crawl from plant to plant transport the internal larva with them. This voluntary local migration of the host is accelerated by an excited female parasite which stirs up the entire colony. Oviposited nymphs may become winged and fly from place to place, carrying with them the parasite larva, but out of twenty-five alate *M. persicae* trapped in the field the larva was found in one case only. Under laboratory conditions, out of 120 alate *M. persicae* collected from breeding glass chimneys, thirty were parasitized, of which eight had the parasite in the egg stage, eleven in the 1st stage, four in the 2nd stage, two had it each in the 3rd, 4th and fully grown stages. In the last two stages the alate were stationary. In the laboratory, out of 4401 sessiles observed there were only 3·4% winged sessiles.

There appears to be very little chance for the parasite to be disseminated through oviposition in alate *M. persicae*, for under laboratory conditions in glass cavity blocks the parasite found great difficulty in ovipositing in an alata. Dissemination, therefore, appears to be more probable through the flight of the adult parasite itself and through localized movements of oviposited apterous *M. persicae*.

7. Effect of the parasite on its host

Since *Aphidius matricariae* is an internal parasite, in the course of its development (egg and larval stages) it brings about both physical and chemical changes in the host.

The parasite in its egg stage does not noticeably influence the host physically. The same may be said of the larva up to the 2nd stage, but as soon as it reaches the 3rd stage it begins to feed through the mouth. First, it feeds on the adipose tissue and older host embryos; later on, younger embryos are attacked.

The digestive system of the host is next to be ingested; this occurs when the larva is fully grown. Thirty-four parasitized *Myzus persicae* were dissected, of which twenty showed larvae in the full-grown stage and fourteen had them in the 4th stage. Of the former, seven showed the digestive system disorganized and thirteen showed it altogether destroyed. Out of the fourteen cases, three showed disorganization of the digestive system, the remaining eleven had the system entirely intact. In the course of routine dissections, it was observed that the 3rd stage larva, even though it was entangled in the loop of the host's alimentary canal, did not damage it. The gut of the host was destroyed 8 hr. before the moribund stage of the host was reached. At this stage the larva feeds feverishly and begins to attack the nervous, circulatory and musculatory systems. The greater part of the respiratory system is left throughout. The host stops reproducing when the parasite larva reaches the 3rd stage; until then it goes on reproducing normally. At this stage dissections show that the whole system is involved and thus reproduction ceases. Forty-four dissections were carried out on parasitized *M. persicae*, out of which thirty-four had parasite larvae in the 4th stage and had no healthy

embryos, in the six cases where the 3rd stage larvae were present the aphides had no healthy embryos, in two cases the larvae were in the 2nd stage and these had two and three healthy embryos respectively, in two cases the larvae were in the 1st stage and each had three healthy embryos.

It appears that when the parasite oviposits in a 3rd instar host the latter does not reach the stage of reproduction. It only follows when oviposition takes place in the 4th instar or in the adult stage of the host. When this is the case, the host lives 5–7 days, even after the cessation of reproduction.

8. SUMMARY

1. *Aphidius matricariae* Hal. is an internal parasite of *Myzus persicae* Sulz.; it was also found parasitizing *M. circumflexus* Buckt. and *Aulacorthum solani* Kalt. (*Myzus pseudosolani* Theob.).

2. The percentage of aphides successfully parasitized increased with an increased number of strikes per host. A single female can lay up to 350 eggs, and one female successfully parasitized 309 *M. persicae*.

3. The female parasite reaches its maximum activity during the 2nd or 3rd day of oviposition, and this activity has no correlation with light intensity.

4. The disappearance of supernumerary larvae due to cannibalism has been observed.

5. The species is arrhenotokous. Mating takes place from 2 hr. after emergence. In the progeny of early matings the females preponderated; in later matings the sex ratio was approximately 1:1.

6. The parasite appears to fly under the same conditions as those generally favourable for aphides. It is generally disseminated by flight of the adult itself and through localized movements of parasitized apterous aphides.

7. The host is unable to reproduce if 'struck' in the 3rd instar or earlier.

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COMPARATIVE EFFICACY OF DIFFERENT CULICIFUGES UNDER LABORATORY CONDITIONS

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Among a very large number of preparations reputed to possess mosquito-repellent properties, only a few are really efficacious. The most commonly used preparations are those prepared with citronella oil. Definite information, however, on the comparative efficiency of many of these preparations is lacking. It was therefore our object to determine the value of different culicifuges under laboratory conditions. The experimental results obtained in this connexion are sufficiently promising to justify their publication, especially because owing to the war the want of an effective and a cheap culicifuge is all the more important.

All mosquitoes used in our experiments were reared in the laboratory from larvae collected in the field, and no experiment was performed on insects which were less than 3 days old; older mosquitoes were rejected.

Mosquitoes after emergence were sustained on raisins and water except for about 18 hr. before the tests were undertaken when food was withdrawn. A batch of mosquitoes generally consisted of not less than fifty (*Anopheles stephensi*) up to many hundreds of female (*Armigeres obturbans*, *Aëdes egypti* and *Culex fatigans*). A batch of mosquitoes when once used for biting experiments was seldom used again during the course of the same day.

The mosquitoes were kept in a feeding cage $13 \times 13 \times 13$ in.. having cloth at the sides with two projecting sleeves and glass at the top.

Although in every instance preliminary observations were made to determine the biting propensity of the mosquitoes, in many doubtful cases where the mosquitoes showed little inclination to approach the treated parts, it was necessary to introduce the untreated arm of another volunteer into the same cage through the opposite sleeve as a control.

The method adopted for testing a fluid preparation was to smear both sides of the hand and forearm with cotton-wool soaked in the testing substance. In order to confirm the results obtained in the preliminary experiments only a limited part of the hand was treated, so that the mosquitoes might be attracted to the untreated parts. It was thought unnecessary to count the number of bites inflicted. The observations were generally done in the day time and if necessary in darkness during February, March and April and they were repeated in August.

The oil of citronella used in our experiments was from 2-year-old stock prepared by Schimmel and Co. and labelled 'Oil of Citronella: Ceylon II'. 'Pyrocide 20' was a standardized extract of pyrethrum manufactured by McLaughlin Gormley King and Co., U.S.A. The other preparations were obtained locally.

We do not think any conclusion on the relative merits of different preparations should be drawn from laboratory tests alone; they should be supplemented by parallel observations in the field. One has to take into consideration that the behaviour of

Preparation used	Period of effectiveness			
	Aëdes egypti	*Armigeres obturbans*	*Anopheles stephensi*	*Culex fatigans*
1. Citronella oil:				
*(a) Very small amount used	20 min.	25 min.	45 min.	1 hr.
*(b) Large amount	45 min.	55 min.	1 hr. 20 min.	2 hr.
2. Citronella oil, ½ part; coconut oil, ½ part:				
(a) Very small amount	25 min.	25 min.	35 min.	40 min.
(b) Large amount	40 min.	40 min.	1 hr. 5 min.	1 hr. 25 min.
3. Citronella oil, 10 parts; Pyrocide 20, 1 : 20 in kerosene, 10 parts; coconut oil, 80 parts:				
(a) Very small amount	20 min.	22 min.	1 hr.	1 hr. 10 min.
(b) Large amount	1½ hr.	1 hr. 40 min.	2 hr.	2 hr. 10 min.
4. Citronella oil, 10 parts; Pyrocide 20, 1 : 20 in kerosene, 20 parts; coconut oil, 70 parts:				
(a) Small amount	35 min.	48 min.	1 hr. 5 min.	1½ hr.
(b) Large amount	2 hr. 10 min.	2 hr. 25 min.	2 hr. 45 min.	3 hr.
5. Citronella oil, 20 parts; Pyrocide 20, 1 : 20 in kerosene, 30 parts; coconut oil, 50 parts:				
(a) Small amount	2 hr. 52 min.	3 hr. 10 min.	4 hr. 5 min.	More than 5½ hr.
1. Lemon-grass oil:				
(a) Very small amount	45 min.	48 min.	1 hr. 5 min.	1 hr. 15 min.
(b) Large amount	1 hr.	1 hr.	2 hr.	2 hr. 15 min.
2. Lemon-grass oil, ½ part; coconut oil, ½ part:				
(a) Very small amount	40 min.	40 min.	48 min.	55 min.
(b) Large amount	1 hr. 10 min.	1 hr. 20 min.	1 hr. 40 min.	2 hr.
3. Lemon-grass oil, 10 parts; Pyrocide 20, 1 : 20 in kerosene, 10 parts; coconut oil, 80 parts:				
(a) Very small amount	1 hr. 10 min.	1 hr. 15 min.	2 hr.	2 hr. 15 min.
(b) Large amount	1½ hr.	1 hr. 40 min.	2 hr. 35 min.	3 hr.
4. Lemon-grass oil, 10 parts; Pyrocide 20, 1 : 20 in kerosene, 20 parts; coconut oil, 70 parts:				
(a) Small amount	2 hr.	2 hr. 10 min.	3 hr. 5 min.	3½ hr.
(b) Large amount	2 hr. 40 min.	2 hr. 52 min.	3 hr. 40 min.	4 hr. 10 min.
5. Lemon-grass oil, 20 parts; Pyrocide 20, 1 : 20 in kerosene, 30 parts; coconut oil, 50 parts:				
(a) Small amount	3 hr. 25 min.	3 hr. 48 min.	4½ hr.	More than 5½ hr.
Pyrocide 20, 1 : 20 in kerosene:				
Very small amount	2½ hr.	2 hr. 35 min.	3 hr. 55 min.	5 hr. 10 min.
Coconut oil:				
Large amount	1 hr.	1 hr. 10 min.	2 hr.	2 hr. 10 min.
Carbon tetrachloride	Nil	Nil	Nil	Nil
Oil turpentine:				
Small quantity	Nil	20 min.	20 min.	40 min.
Large quantity	30 min.	40 min.	45 min.	1 hr. 5 min.
Naphtha	No repellent properties			
Benzene	No repellent properties			
Creosote: 5% in liquid paraffin:				
Small quantity	Nil	Nil	Nil	Nil
Kerosene oil:				
(a) Small amount	Nil	Nil	10 min.	15 min.
(b) Large amount	20 min.	35 min.	1–15 min.	1 hr. 40 min.
Pyrethrum ointment:				
In vaseline, 1 : 12	10 min.	50 min.	1 hr. 25 min.	1 hr. 35 min.
Methyl salicylas:				
In liquid paraffin, 1 : 8	Nil	Nil	15 min.	20 min.
In kerosene oil, 1 : 8	12 min.	12 min.	25 min.	35 min.
Sesame oil:				
Small amount	5 min.	10 min.	1 hr.	2½ hr.

* (a) Only sufficient mixture was used in all experiments to spread the thinnest possible film on the skin.
(b) The film was put sufficiently thick to be visible.

mosquitoes in the feeding cage is not the same as occurs in nature. It is true that *Aëdes egypti* bite man readily and prefer to feed on human blood, and therefore laboratory experiments with this species may indicate to a great extent the true value of a culicifuge when used in the household to protect one from their bite. But it must be remembered that the efficacy of a culicifuge is entirely dependent on its volatilization and this is greatly helped by breeze. In the feeding cage there is a natural retardation of the process. Relying on laboratory studies alone it can be stated that a mixture of coconut oil and pyrethrum should prove an efficient culicifuge. This is soothing to the skin, and also has the advantage that it is cheap.

Whether the preparation should be of a solid or fluid nature is open to question. Bacot & Talbot (1919) are of the opinion that the essential ingredients should be made up with wax, and that fluid preparations are not only wasteful in application but are inconvenient also. Whether vaseline or soft wax be the chosen vehicle, their application to the skin in the tropics during the rainy season and autumn causes so much discomfort as to make them very distasteful to many people.

It is necessary to add that the smell of kerosene is completely masked by citronella oil or lemon-grass oil when a few drops of the latter are added to the former.

Citronella oil or lemon-grass oil, when applied strong, causes an increase of vascularity of the parts; this is followed by an excess of perspiration.

Pyrocide 20 or any other strong liquid preparation of pyrethrum is fatal to mosquitoes when there is an intimate contact between the two, e.g. when a mosquito sits on a part treated with pyrethrum; its vapour also can paralyse the activities of the insect.

REFERENCE

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ON THE PARASITES OF SILVER FOXES ON SOME FARMS IN THE SOUTH-WEST

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(With 5 Figures in the Text)

Introduction

It had been hoped to follow the survey outlined here by a series of controlled experiments designed to elucidate (i) the more important features in the life cycles of, in particular, the nematode parasites of the silver foxes, and (ii) the effects of the various infestations on the foxes, and methods of controlling them, or of ameliorating the damage done. In fact, in September 1939, we were on the point of obtaining, by the kind courtesy of several of the fox farmers with whom we had been working, a number of experimental animals. With the advent of war, however, it became apparent that this work must be at least temporarily abandoned. The results of the survey seemed to us to be sufficiently interesting in themselves, and to provide a basis for future observations, should they at any time become possible. We have therefore decided to publish them as they stand.

The figures analysed in the following pages have been obtained during the course of an investigation into the diseases of silver foxes and of methods of controlling or preventing their outbreak. Carcasses and samples of faeces have been obtained from fourteen farms altogether. In most cases the material was first obtained as the result of a specific request to the owner, and some selection of the carcasses to be examined was made with a view to obtaining a representative sample from the farm. In addition to these animals, however, other carcasses were sent in with requests for information as to the cause of death. These animals were examined by the same routine methods as the others for which request had been made. In some cases the only animals obtained from a farm have belonged to the second category, the farm having been for various reasons omitted from our initial survey.

In all 154 carcasses have been examined, and in most cases the worm populations related to the worm egg counts obtained by the Stoll dilution count method. Of this total twenty-seven animals were selected during the 1937 season, and their faeces examined at about fortnightly intervals over a period of up to 2 months before they were pelted. As will be seen from the analysis of the figures presented later, the worm egg counts fluctuated violently and in a very irregular manner, and it proved impossible to discover any quantitative relation between the counts from the faeces and the worm populations in the carcasses.

In addition to the silver foxes, we have examined nine adult and four cub red foxes, in order to determine how far it is possible that the silver-fox farms may be infected from wild red foxes living in their neighbourhood. Some of these carcasses have been obtained locally, but as most of the area is hunted it has not been possible to obtain an adequate number to provide a true indication of the extent to which the wild species is parasitized. We have had to rely on chance deaths in traps, etc. The local foxes have therefore been supplemented by animals obtained from near Oswestry, where, in the absence of hounds, the foxes are shot.

It remains only to be added that in addition to the examination of the animals mentioned in the foregoing paragraphs a large number of faeces samples have been examined from animals which have been retained in the breeding herds of various farms. We are in a position therefore to state that worm parasites are extremely prevalent among the silver foxes in the south-west. It is very rare to find an animal which is entirely free, and the majority of them are heavily infested with one and often more of the common species of worm we have found.

Material and methods

The procedure on most farms is to pelt in November to early January those cubs which are not considered satisfactory for breeding. The cubs having been littered in the previous spring, from late March to early May, most of them are about 8 or 9 months old when pelted. In addition, those animals which have proved unsatisfactory in breeding, after one or more seasons may be weeded out, giving classes of 21 and 33 months, etc., described here as two-year, three-year and up. The remainder of the foxes examined consist of animals which have died suddenly, during or outside the pelting season, or of dogs run as 'teasers' to the breeding vixens, and pelted after the mating season in February to March. They include comparatively few older animals from 1 year upwards, but a large class of young cubs ranging from a few days to about 5 months of age. Wherever possible a record of the age, supposed cause of death, and of the life history of each fox has been obtained from its owner. Owing to human fallibility there is, however, a fairly large group of animals about whose age and antecedents very little information has been forthcoming.

The animals have been obtained as soon as possible after death, as a rule not more than 24 hr. after, but in a few cases as long as 72 hr. had elapsed. On arrival the carcass was opened, the trachea and lungs, stomach, intestines and rectum, liver, urinary bladder and kidneys removed and separated. Scrapings were taken from each ear and examined in caustic potash for ear-mites, while, in the few cases where unpelted carcasses were sent in, the skin was carefully gone over for ectoparasites.

The trachea was severed at its base from the bronchi, opened by a longitudinal cut, and pinned out under water on a black surface. After all the adhering worms had been removed the water covering it was passed through a fine sieve and the sievings examined for any worms which had escaped notice. The lungs were examined by slitting the bronchi, bronchial tubes, etc., by means of a fine ball-pointed canaliculus knife. After removal of any worms seen the whole lung was washed under the tap into a bowl, and then rinsed and squeezed gently into the same bowl. Any worms so washed out of the lungs were recovered by sieving the water and examining the sievings. An important proportion of the male *Crenosoma* were recovered by this means.

The stomach was separated from the intestine, opened, and its contents washed into a bowl and carefully broken up and examined for parasites. The intestine, separated from the rectum, was squeezed empty and then slipped on to a tap and thoroughly washed out into a bowl. The squeezed contents and the wash-water were thoroughly agitated together and then sieved. It was found that all the worms in the intestine could be recovered by this procedure, slitting the intestine and examining its walls revealing the presence of no more worms adhering to the mucosa.

From the rectum the required quantity of faeces was weighed out for the Stoll dilution

count, and the remainder was then thoroughly broken up in water in order to recover any *Uncinaria* present in this section of the gut.

The bladder was opened and pinned out under water on a black surface and any *Capillaria plica* removed. Each kidney was split longitudinally and its pelvis carefully examined for specimens of this same species of worm.

THE WORM PARASITES FOUND

Of the Platyhelminthes we have found only *Taenia pisiformis* (Bloch, 1780) during the course of our investigation. This cestode is by no means uncommon on those farms which use rabbit meat. It was rarely found in any large numbers, this undoubtedly being due to the care with which the rabbit carcasses are examined before being used. The numbers found in the wild red foxes examined were very much greater in many instances. Various writers (summarized by Freund, 1930) have recorded a number of other cestodes and several trematodes from the silver fox and from red foxes. In spite of most careful searching of the gut contents in every case we have been unable to record the presence of any of these species in the animals we have received.

Of nematoda we have found five species commonly present, namely:

Trichuridae:

Capillaria plica (Rudolphi, 1819).

Eucoleus (*Capillaria*) *aerophilus* (Creplin, 1839).

Metastrongylidae:

Crenosoma vulpis (Dujardin, 1845).

Uncinaria stenocephala (Railliet, 1884).

Ascaridae:

Toxocara canis (Werner, 1782).

In addition to these five species we have a single record of one male *Toxocara mystax* (Zeder, 1800) in a silver fox. The virtual absence of this species from the foxes is significant in view of the fact that the practice of rearing cubs on a cat when the vixen fails is quite common. *T. mystax*, together with *Toxascaris leonina* (Linstow, 1902), is common in the kittens reared with the cubs in some instances. It seems certain therefore that the foxes are endowed with strong powers of resistance to both these nematodes. It is also noteworthy that no single occurrence of *Ancylostoma caninum* Ercolani, 1859, was recorded.

In addition to the helminth parasites found the following arthropods were noted:

Acarina:

Otodectes cynotis (Hering, 1828).

Demodex folliculorum Simon, 1842.

Pentastomida:

Linguatula serrata Frölich, 1789.

Insecta:

Ctenocephalus canis (Curtis, 1826).

Spilopsyllus cuniculi Dale, 1878.

Otodectes was found to be prevalent on almost all farms when the investigation was commenced, but as a result of our diagnoses, and the consequent routine dressing of the ears instituted on all the farms, by the end of the period this mite had become notably

less frequent, and on some farms was almost absent. Of the total of foxes examined sixty-six were carrying *O. cynotis*, forty-three of them being heavily infested. One case only of follicular mange was observed, but occasional individuals of *Demodex* occurred in the ear scrapings. *Linguatula* was only found once, as an immature form in the gut of a red fox.

The only protozoan parasite found was the coccidian *Eimeria vulpis* Galli-Valerio, 1928. Although at times the cause of serious cub losses, this parasite was not generally common among adult foxes.

There is little doubt that in this area by far the most widespread, persistent, and harmful parasites of the silver foxes are the nematodes listed above, and our observations have been concentrated upon them.

The figures from the silver foxes have been analysed in two groups. Group I is comprehensive of the entire number of silver foxes examined, while in group II are those foxes, removed from group I, of which both the carcass and a series of droppings have been obtained and examined.

In group I further subdivisions have been made, and the results are presented in Tables 1–4. Table 1 details those foxes which were described by their owners as healthy and of good pelt. These animals were not always as healthy as expected. Table 2 represents animals which have been pelted but have shown during their lives various symptoms of ill health and backwardness, either in general physique or in a poor-quality pelt, or in both. Table 3 comprises an anomalous group of animals, some of which were received without any information as to age, health, etc., while others have died suddenly from various causes, such as leptospiral jaundice, a form of encephalitis, torsion of the stomach, suffocation by worm capsule, etc. Lastly, in Table 4 are included the cubs of the 1937 and 1938 breeding seasons which died before the corresponding pelting seasons in the autumn following their births, at ages ranging from 1 day to about 5 months. All the animals in this group died suddenly from some disease or from some abnormal cause, and should therefore be considered with the unhealthy group. The figures have been analysed by various methods with a view to discovering (i) the degree to which various of the parasites tend to be localized on different farms, (ii) the possibility of positive or negative correlations between one species of worm and another, (iii) the age of foxes at which the incidence of parasites is first noticed, (iv) any indication of either acquired or age resistance to any of the nematodes, (v) the variation in the relationship between eggs or larvae in the faeces, and the worms present in the organs of the host fox, (vi) the sex ratios of the worms.

The distribution of parasites on the different farms

In Tables 5 and 6 are detailed the observations obtained from fifteen different farms. Table 5 relates to adult foxes and Table 6 to cubs. For the purposes of this paper foxes bred in the spring and killed for pelting in the following winter, at an age of 7–9 months, are regarded as being in the adult group. The term 'cub' is restricted to animals dying before reaching 7 months of age.

It will be seen that the numbers of animals obtained from the different farms ranges from twenty-nine (adults and cubs) to a single individual. Where the sample examined is so small as this last figure no value can be attached to it as indicator of the conditions on the farm, and in view of this only those farms from which a reasonably large sample

Table 1. *Distribution of parasites. Healthy foxes*

Table 1. *Distribution of parasites. Healthy foxes*

Notes. (i) = number of adult worms found in the carcass; figures in brackets indicate immature worms not considered in computing egg/female ratios. (ii) = larvae or eggs per gram of faeces. (iii) = larva or egg/female ratio. Under *C. plica* figures in brackets indicate the numbers of this worm found in the kidneys. n.f. = no faeces. f.a. = females absent.

			Wt.		*C. vulpis*			*E. aerophilus*			*T. canis*			*U. stenocephala*			*T. pisiformis*	*C. plica*
Fox	Age	Sex	lb.	oz.	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii		
1	20 m.	m.	9	10	0	0	—	1	50	50	4	2900	967	1	0	—	0	9 (2)
2	20 m.	f.	7	15	0	0	—	1	0	—	0	0	—	5	166	83	0	5
17		m.	13	1	0	0	—	0	0	—	0	0	—	1 (1)	0	0	0	3
26	4 y.	f.	8	7	0	0	—	1	0	0	1 (1)	0	0	12	100	17	2	7
27	4 y.	m.	9	7	0	0	—	12	5,000	625	15 (4)	2633	329	5	466	233	0	40
28	3 y.	f.	9	11	0	0	—	1	100	100	3	0	0	1	0	—	0	25
30	9 m.	m.	6	0	25	100	10	4	2,633	2,633	3 (3)	0	0	333	12,466	50	1	5
33	cub	m.	7	3	0	0	—	78	533	13	(5)	0	—	49	1,400	40	9	60
34		f.	7	8	0	0	—	79	0	0	6 (10)	400	67	42	300	13	1	59 (7)
42		f.			44	100	—	0	0	—	7	200	—	34	500	—	0	32
47	31 m.	m.	9	4	2	0	0	4	50	12	0	0	—	0	0	—	1	0
48	19 m.	m.	9	14	26	100	7	20	100	9	6	300	75	5	100	25	0	35 (2)
49	15 m.	f.	8	12	0	0	—	10	0	0	0	0	—	68	600	13	0	24
50	8 m.	m.	10	3	12	66	13	2	33	f.a.	6 (6)	900	300	25	350	29	0	30
51	8 m.	m.	8	10	0	0	—	77	166	6	4 (1)	133	133	18	933	78	0	5
52	8 m.	f.	7	4	10	233	23	4	0	0	3 (4)	166	83	33	2,700	123	0	68
53	8 m.	f.			0	0	—	0	0	—	1	400	400	19	200	17	0	40 (4)
54	8 m.	m.			23	200	11	36	150	7	10	1550	194	69	1,300	33	0	44 (11)
58		m.	9	8	9	50	7	15	0	0	2 (4)	0	—	15	100	9	0	76
64	32 m.	f.	8	14	(2)	0	—	0	0	—	2	200	f.a.	9	800	134	0	10
65	32 m.	f.			11	n.f.	n.f.	1	n.f.	n.f.	0	n.f.	n.f.	4	n.f.	n.f.	0	6
66	8 m.	m.	9	1	40	0	0	0	1,500	f.a.	42	0	0	0	0	—	0	0
67	8 m.	m.	9	8	1	0	0	0	1,600	f.a.	7	50	12	0	0	—	0	23
68	8 m.	m.	9	15	81	1900	34	1	15,100	15,100	18 (6)	1700	283	0	0	—	0	27
75	9 m.	m.	9	14	2	0	0	0	0	—	2 (1)	1033	516	11	300	50	0	0
86	20 m.	f.	8	9	0	0	—	1	0	—	0	0	—	(1)	0	—	0	9
87	8 m.	m.	10	1	0	0	—	0	0	—	0	0	—	8	100	20	0	0
93	6½ y.	f.	10	3	0	0	—	6	0	0	0	0	—	21	0	0	0	12
94	8 m.	f.	7	15	22	133	9	14	900	180	4 (1)	166	83	52	400	13	0	13 (1)
95	8 m.	m.	9	5	10	0	0	29	66	5	2 (1)	266	f.a.	32	366	18	0	6
104		m.	11	0	0	0	—	28	333	22	(2)	0	—	15	600	67	0	21
105		m.	9	0	51	1200	39	1	1,500	1,500	1 (2)	233	233	14	766	77	9	10
106		f.	9	8	0	0	—	4	400	200	0	0	—	34	1,100	44	0	9
107		f.	9	7	0	0	—	7	33	11	0	0	—	5	900	211	1	6
108		f.	7	9	13	0	0	7	0	0	0	0	—	58	0	0	0	6
109		f.	8	11	0	0	—	0	0	—	0	0	—	12	233	39	0	0
110		m.	8	8	1	0	—	1	33	f.a.	3 (3)	0	0	88	500	11	0	16
111		f.	7	8	10	n.f.	n.f.	1	n.f.	n.f.	(1)	n.f.	n.f.	48	n.f.	n.f.	0	2
112		m.	8	6	52	600	14	48	2,000	87	6 (3)	0	0	31	666	35	0	13 (1)
113	8 m.	f.	9	5	12	66	11	3	66	22	13 (3)	100	25	40	566	26	0	12

Table 1 (*continued*)

Fox	Age	Sex	Wt. lb.	Wt. oz.	*C. vulpis* i	*C. vulpis* ii	*C. vulpis* iii	*E. aerophilus* i	*E. aerophilus* ii	*E. aerophilus* iii	*T. canis* i	*T. canis* ii	*T. canis* iii	*U. stenocephala* i	*U. stenocephala* ii	*U. stenocephala* iii	*T. pisiformis*	*C. plica*
114	8 m.	f.	7	15	51	66	3	3	33	16	5 (1)	66	f.a.	33	166	7	0	21 (1)
115	2½ y.	f.	7	12	0	0	—	0	0	—	0	0	—	1	133	133	0	0
116	8 m.	m.	10	2	84	166	4	1	2,766	f.a.	46 (25)	4766	183	0	33	f.a.	0	63
117	8 m.	m.	8	8	13	133	13	34	200	13	33 (20)	0	0	0	0	—	0	26
118	8 m.	f.	10	0	84	733	12	0	1,400	f.a.	13 (13)	0	0	0	0	—	0	15 (4)
119		f.	8	0	10	100	17	5	0	0	1	0	—	2	166	166	0	13 (1)
120		m.	8	10	2	0	0	10	2,066	295	2 (1)	1700	f.a.	0	0	—	0	0
121		m.	8	11	0	0	—	11	166	42	2 (3)	200	200	10	300	43	0	10
122	8 m.	f.	8	4	1	0	0	2	1,500	750	18 (45)	0	0	2	66	33	0	12
123		m.	9	7	0	0	—	1 (2)	0	—	2 (1)	2100	2100	33	366	24	29	0
124		f.	8	3	0	0	—	3	0	0	0	0	—	22	66	4	0	5
125	8 m.	m.	7	4	0	n.f.	n.f.	1	n.f.	n.f.	0	n.f.	n.f.	122	n.f.	n.f.	1	8
151		m.	9	12	2	n.f.	n.f.	8 (27)	n.f.	n.f.	0	n.f.	n.f.	7	n.f.	n.f.	0	6
152		m.	10	4	0	0	—	16 (28)	100	9	3 (2)	166	83	8	266	44	0	137 (5)
153		m.	10	8	11	166	28	27 (22)	733	37	3 (2)	0	0	11	966	138	5	76
154		m.	9	0	63	400	10	37	600	37	(6)	0	—	15	33	4	0	34 (1)

Table 2. *Distribution of parasites. Unhealthy foxes*

Notes. As in Table 1. *Eimeria vulpis* was found in foxes 7 and 76.

5	8 m.	f.	9	12	0	0	—	22	0	0	(1)	0	—	6	0	0	0	57
6	8 m.	m.	12	0	0	0	—	76	100	3	7	100	50	64	1400	38	0	16
7	8 m.	f.	7	14	0	0	—	210	466	4	5 (7)	0	0	47	1133	47	7	0
14	20 m.	m.	9	0	0	0	—	1 (2)	0	0	0	0	—	0	0	—	0	2
16	8 m.	m.	8	12	0	0	—	95	13,350	267	0	0	—	25	2600	130	0	62
31	cub	m.	8	2	0	0	—	10	2,700	338	6 (3)	0	0	40	2800	93	0	40
32	cub	m.	7	1	0	0	—	6	9,333	2333	8 (21)	33	5	70	5966	50	0	77
37	8 m.	m.	8	2	254	3000	19	1	100	100	(1)	0	—	27	500	23	0	1
38	8 m.	m.	8	1	363	4133	18	0	0	—	0	0	—	57	800	33	0	3
43		f.			1	100	100	26	1,200	71	(274)	0	—	30	300	—	0	70 (10)
56		m.			8	150	37	66	2,650	65	0	0	—	19	550	55	0	19 (2)
57		f.			50	800	19	270	4,200	25	(10)	0	—	67	3000	81	0	85 (1)
69	8 m.	m.	10	8	46	200	6	2	233	116	27 (15)	66	5	0	0	—	0	47
72	13 m.	f.	11	0	13	0	0	8	666	167	0	400	f.a.	41	2500	104	0	16
73	12 m.	m.	10	1	4	n.f.	n.f.	0	n.f.	n.f.	0	n.f.	n.f.	45	n.f.	n.f.	0	0
74	9 m.	m.	10	9	3	20	10	0	0	—	8 (4)	720	180	32	740	41	4	0
76	9 m.	m.	8	7	1	0	0	1	0	—	3	500	250	134	3450	44	24	0
77	9 m.	m.	9	1	159	266	3	9	133	27	15 (2)	1200	240	35	333	14	0	0
78	9 m.	m.	11	0	35	100	6	0	0	—	1	100	100	30	200	12	0	0
79	8 m.	m.	8	0	208	3550	25	1	0	0	8	850	283	31	1850	103	1	0
85		m.	7	14	0	0	—	5	133	27	2 (1)	33	33	59	1333	34	0	14
88		f.	9	0	0	0	—	0	0	—	0	0	—	5	100	50	0	0
89		m.	7	2	0	0	—	(1)	0	—	0	0	—	15	933	104	0	6

88		f.	9	0	0	0	—	0	0	—	0	0	—	5	100	50	0	0
89		m.	7	2	0	0	—	(1)	0	—	0	0	—	15	933	104	0	6

Table 3. *Foxes, previous history unknown, or dying suddenly*

Notes. As in Table 1.

			Wt.		*C. vulpis*			*E. aerophilus*			*T. canis*			*U. stenocephala*			*C. plica*
Fox	Age	Sex	lb.	oz.	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	
3	12 m.	f.	7	15	0	0	—	0	0	—	0	0	—	95	800	13	50
4	1 y.	m.	9	4	0	0	—	3	300	100	0	0	—	13	200	29	42
13		m.	10	1	0	0	—	1	0	—	0	0	—	0	0	—	15
15	8 m.	m.			29	100	5	5	0	0	0	0	—	18	400	40	4
18		m.	9	1	0	0	—	42 (8)	0	0	0	0	—	0	0	—	53
19		m.	9	0	3	n.f.	n.f.	(1)	n.f.	n.f.	0	n.f.	n.f.	5	n.f.	n.f.	9
20		f.	8	14	0	0	—	101	66	1	0	0	—	10	33	5	14 (1)
21		f.	8	8	0	0	—	2	0	0	0	0	—	0	0	—	1
25	cub	f.	6	15	8	33	7	13	0	0	0	0	—	5 (3)	0	0	78
29	15 m.	m.			0	0	—	0	0	—	0	0	—	11	133	17	0
39	8 m.	f.	7	11	23	933	62	0	66	f.a.	0	0	—	172	3,233	30	20
40	8 m.	f.			32	250	10	3	0	0	1	200	200	162	3,450	35	29
41	7 m.	f.	8	9	61	1666	42	20	200	13	4	366	366	113	533	7	11 (1)
55		f.			12	400	33	84	200	5	0	0	—	10	200	—	50
59		f.	9	1	0	n.f.	n.f.	0	n.f.	n.f.	1	n.f.	n.f.	23	n.f.	n.f.	Num.
60		m.			1	0	—	30	200	—	17	1400	—	30	1,900	—	0
61		f.	6	15	10	n.f.	n.f.	0	n.f.	n.f.	0	n.f.	n.f.	57	n.f.	n.f.	1
70					112	4900	70	13	11,655	1295	12 (2)	300	43	8	1,650	275	33 (1)
80					49	1550	49	0	0	—	4 (1)	550	183	56	300	8	0
84		f.	8	13	0	0	—	2	33	33	5 (1)	66	33	8	66	22	9
92		m.	9	8	0	0	—	17	0	0	2	200	f.a.	2	0	0	21 (1)
141		m.	7	11	3	0	0	58	9,600	320	0	0	—	168	18,100	201	30
145		f.			0			0			161 (180)			0			0
146	3 y.	f.			0			0			0			0			0

Table 4. *Distribution of parasites. Cubs dying out of pelting season*

Notes. As in Table 1.

			Wt.		*E. aerophilus*			*T. canis*			*U. stenocephala*			*Eimeria vulpis*
Fox	Age	Sex	lb.	oz.	i	ii	iii	i	ii	iii	i	ii	iii	
83	18 d.	m.	0	5½	0	0	—	(6)	0	—	0	0	—	0
9	46 d.	f.	1	5	0	233	f.a.	29	89,133	3,745	1	0	0	0
10	34 d.	f.	0	14	0	0	—	5 (1)	35,200	11,733	0	0	—	Pres.
11	33 d.	f.	0	14½	0	0	—	179	1,933	14	0	0	—	Pres.
12	29 d.	m.	0	9½	0	0	—	4	16,766	16,766	0	0	—	0
46	69 d.	m.	2	6	0	0	—	0	0	—	2	0	0	0
63	10 w.	f.	1	14	0	0	—	11 (21)	500	167	18	500	62	2,300
81	2 m.	f.	1	12	0	0	—	0	0	—	1	0	0	0
82	2 m.	m.	2	6	0	0	—	0	0	—	16	1,200	109	0
8	14½ w.	f.	3	7	0	0	—	2 (3)	0	—	0	0	—	0

Table 4 (*continued*)

Fox	Age	Sex	Wt. lb.	Wt. oz.	*E. aerophilus* i	*E. aerophilus* ii	*E. aerophilus* iii	*T. canis* i	*T. canis* ii	*T. canis* iii	*U. stenocephala* i	*U. stenocephala* ii	*U. stenocephala* iii	*Eimeria vulpis*
22	4 m.	m.	6	14	202	3966	38	0	0	—	27	2,800	155	0
23	4 m.	m.	8	14	250	1300	10	0	0	—	5	1,000	500	0
24	4 m.	f.	9	4	170	n.f.	n.f.	0	n.f.	n.f.	42	n.f.	n.f.	—
35	5 m.	m.	8	12	0	100	f.a.	1	0	—	40	200	9	0
36	14 w.	m.	6	8	0	n.f.	n.f.	1 (1)	n.f.	n.f.	210	n.f.	n.f.	—
44	87 d.	f.	2	11	0	n.f.	n.f.	1 (20)	n.f.	n.f.	4	n.f.	n.f.	—
45	87 d.	f.	2	9	0	n.f.	n.f.	(16)	n.f.	n.f.	3	n.f.	n.f.	—
62	3½ m.	m.	2	10	0	0	—	2 (14)	0	0	362	1,700	9	0
71	1–2 m.	m.	3	11	0	566	f.a.	(85)	0	—	0	0	—	0
90	5 m.	m.	8	4	0	0	—	0	0	—	58	1,266	44	0
91	5 m.	m.	9	6	0	0	—	1	133	133	79	766	19	0
96	1–2 m.	m.	2	5	48	1400	38	12 (1)	2,600	1,300	91	13,000	271	3,300
97	1–2 m.	m.	2	7	0	0	—	3 (2)	9,150	9,150	4	50	17	100,000
98	9 w.	f.	1	13	0	0	—	0	0	—	111	3,200	48	0
99	1–2 m.		1	15	0	0	—	(3)	0	—	22	4,300	391	0
100	1–2 m.	f.	2	7	0	0	—	(1)	650	f.a.	8	550	183	450
101	1–2 m.	f.	2	6	0	n.f.	n.f.	3	n.f.	n.f.	995	n.f.	n.f.	Few
102	1–2 m.	m.	2	11	0	0	—	2	1,000	1,000	893	41,000	132	27,600
103	1–2 m.	f.	1	11	0	0	—	22	10,400	1,486	20	1,700	131	0
126	3 w.	m.			0	0	—	(1)	0	—	0	0	—	15,000
127	5–6 d.	f.			0			0			0			0
127*a*	5–6 d.	f.			0			0			0			0
128	1–2 w.	f.			0			(6)			0			0
129	4 w.	m.	0	4	0	n.f.	n.f.	13 (5)	n.f.	n.f.	0	n.f.	n.f.	—
130	4 w.	m.			0			0			0			0
131														
132														
133	1 d.	m.			0			0			0			0
134		f.												
138														
139														
135	53 d.	f.	1	7	0	0	—	(2)	0	—	0	0	—	Few
136	1–2 m.	m.			0			11 (2)			15			0
137	1–2 m.	f.			0			0			0			0
140	6 w.	f.	1	6	0			104			0			0
142	5 w.	m.	0	12	0			24			0			0
143	5 w.	f.	0	13	0			22	8,325		0			129,536
144	3 m.	f.	3	9	0			200			9			0
147	19 w.	f.			290			(4)			2			0
148	19 w.	f.			1000			0			0			0
149	20 w.	f.			Very num.			5			0			0
150	20 w.	f.			Very num.			2 (5)			0			0

150	20 w.	f.	Very num.			2 (5)												0	0	

Table 5. *Distribution of parasites on farms. Adult foxes*

Notes. (i) = percentage of foxes infested. (ii) = average number of parasites per fox. (iii) = average larva or egg/female ratio.

			C. vulpis			*E. aerophilus*			*T. canis*			*U. stenocephala*			*C. plica*		*T. pisiformis*		*E. vulpis*
Farm	No. ans.	*O. cynotis*	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	i	ii	i
1	9	66·7	0	0	0	88·0	37·0	5·1	55·6	3·0	533·3	88·9	25·9	26·4	88·9	24·0	11·1	0·8	11·1
2	18	27·8	38·9	9·3	18·8	94·4	26·4	86·9	27·8	1·3	79·8	83·3	10·4	65·1	100·0	29·5	22·2	0·9	16·7
3	20	75·0	80·0	15·5	13·9	85·0	35·7	28·1	75·0	5·7	209·1	95·0	31·8	36·1	95·0	27·2 +	0	0	0
4	11	36·7	90·9	43·2	26·7	54·5	4·7	1230·5	81·8	25·4	73·0	36·7	2·0	201·2	81·8	22·7	0	0	0
5	13	38·5	30·8	3·8	10·0	76·9	15·5	189·0	69·2	7·4	113·6	100·0	66·5	47·0	84·6	26·3	23·1	0·8	0
6	7	85·7	100·0	111·1	21·4	57·1	7·1	43·5	71·4	41·0	29·8	100·0	85·0	26·2	100·0	23·7	0	0	14·3
7	9	88·9	100·0	63·8	19·2	44·4	2·1	79·9	77·8	6·0	223·0	100·0	57·2	43·2	11·1	1·8	33·3	3·2	11·1
8	6	0	50·0	2·2	12·5	100·0	5·8	194·9	100·0	13·5	166·6	83·3	13·3	39·4	83·3	9·7	0	0	16·7
9	5	60·0	0	0	0	80·0	1·6	0	20·0	0·6	2100·0	100·0	37·2	15·2	60·0	4·4	40·0	6·0	0
10	2	0	0	0	0	50·0	0·5	—	0	0	0	100·0	10·0	93·9	50·0	39·0	0	0	0
11	1	100·0	0	0	0	100·0	331·0	—	0	0	0	0	0	0	0	0	0	0	0
12	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 6. *Distribution of parasites on farms. Cub foxes*

Notes. As in Table 5.

			C. vulpis			*E. aerophilus*			*T. canis*			*U. stenocephala*			*C. plica*		*T. pisiformis*		*E. vulpis*
Farm	No. ans.	*O. cynotis*	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	i	ii	i
1	7	0	0	0	0	0	0	0	100·0	61·1	841·0	14·3	0·1	0	0	0	14·3	0·1	42·9
2	11	0	18·2	0·4	33	63·6	174·0 +	22·9	27·3	1·5	—	36·4	6·9	190·0	27·3	2·7	9·1	0·9	0
3	5	40·0	40·0	0·8	11	20·0	11·6	320·0	40·0	9·4	167·0	60·0	109·6	72·7	20·0	6·0	0	0	40·0
4	5	0	0	0	0	0	0	0	40·0	20·6	—	0	0	0	0	0	0	0	0
5	4	0	0	0	0	0	0	0	100·0	12·2	—	50·0	62·5	9·5	25·0	1·7	0	0	25·0
6	4	100·0	0	0	0	0	0	0	75·0	9·7	—	75·0	2·2	—	0	0	0	0	25·0
7	3	66·7	0	0	0	0	0	0	33·3	2·0	—	66·7	5·7	100·0	0	0	33·3	0·3	0
9	1	0	0	0	0	0	0	0	100·0	6·0	—	0	0	0	0	0	0	0	0
10	2	0	0	0	0	0	0	0	50·0	0·5	133·0	100·0	68·5	29·9	0	0	50·0	1·0	0
11	2	0	0	0	0	0	0	0	100·0	2·0	—	100·0	15·0	303·1	0	0	0	0	50·0
13	6	16·7	16·7	0·3	0	0	0	0	83·3	39·7	1140·0	83·5	325·3	69·3	0	0	0	0	33·3
14	2	0	0	0	0	50·0	24·0	37·8	100·0	9·0	3917·0	100·0	47·5	255·9	0	0	100·0	2·5	100·0
15	1	0	0	0	0	0	0	0	100·0	111·0	48·5	100·0	111·0	48·5	0	0	0	0	0

has been obtained, whether of cubs or of adults or of both, have been used for the purposes of the comparison.

In Fig. 1 are plotted the percentages of adult foxes infested, and the average infestations per fox, with the five common nematode worms indicated in the preceding section. These figures relate to nine farms, from each of which at least five adult foxes have been obtained. It will be seen that in all cases the degree of infestation varies widely between one farm and another. The most striking distribution is offered by *Crenosoma vulpis*, which is sparse or absent in the foxes from five farms, but common on the other four. It has been shown (Wetzel & Müller, 1935) that this worm depends for the completion of its life history on certain species of slugs and snails as secondary hosts. Now of the farms which are comparatively free from *Crenosoma*, four, numbers 1, 2, 5, and 8, are situated on the acid, peaty soils of Dartmoor, while from the fifth, farm 9, only the very small total of five foxes has been examined. The remaining four farms, numbers 3, 4, 6, and 7, are on less acid soils, while farms 6 and 7, with the heaviest *Crenosoma* infections, are on Greensand and Culm measures respectively. Boycott (1934) has demonstrated the existence of a marked inverse relationship between soil acidity and land molluscs, and we believe that the character of the soil accounts to a very great extent, if not completely, for the distribution of *Crenosoma* among the farms of the area.

No farm is entirely free from any of the remaining four species of worm. *Toxocara canis* is probably least in importance. Although the proportion of foxes which carry it is relatively high on most farms the average number of worms in each fox is uniformly low, except on two farms where it reaches twenty-five and forty-one respectively. The figures we have obtained do not, however, represent the natural status of the worm. It is the most easily expelled of the fox worm parasites, and on all farms routine pilling is carried out against it, it being thereby kept at a low level. Were it not for this it is probable that it would be almost universally present and in large numbers. The high incidence at an early age in many young cubs before they are old enough to be pilled, indicates that without due care and precautions it would be the cause of serious losses on most farms.

The hookworm, *Uncinaria stenocephala*, is highly prevalent on all but one of the farms under consideration. Although there are pills on the market which are designed for the expulsion of this worm, and which are used by most farmers, it is obvious from comparison of the figures given for this species with those for *Toxocara* that the pilling is by no means as effective against the hookworm as it is against ascarids. The factors which may account for the relative sparseness of hookworms on farm 4 are obscure. The incidence of the other parasites is relatively high, and it does not seem that good management may account for it, especially in view of the large numbers of ascarids in the same animals. The foxes are from the same strain as are most of those in the area, and it is therefore unlikely that genetic resistance may account for their apparent immunity. It is possible that the original stock introduced on to the farm was freer than most foxes from the worm, but as the farm has been in existence for about 10 years, it is not at all likely that this condition may still be maintained without the operation of some environmental factor inimical to the worm.

The bladder worm, *Capillaria plica*, is rather more uniformly common than the other species, but on farms 7 and 9 is much less prevalent than elsewhere. This worm un-

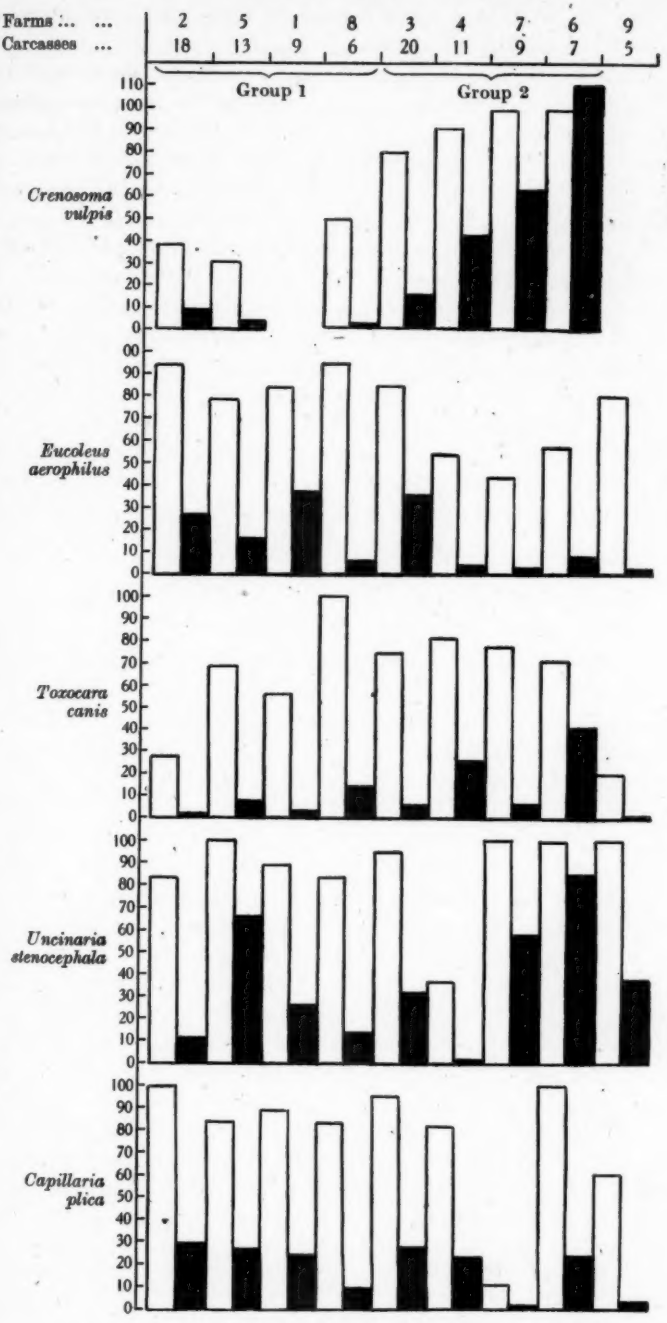
Farms 2 5 1 8 3 4 7 6 9
Carcasses ... 18 13 9 6 20 11 9 7 5
Group 1
Group 2
= % foxes infested
= Average number of worms per fox
Crenosoma vulpis
Eucoleus aerophilus
Toxocara canis
Uncinaria stenocephala
Capillaria plica

Fig. 1. Distribution of worms on some farms. Adult foxes.

doubtedly requires special investigation, as it is believed by many fox owners to be the cause of a great deal of the trouble which some foxes experience in mating.

Eucoleus aerophilus[1] is present on all the farms, a high proportion of the foxes generally being infested, sometimes very heavily. But whether measured by the numbers of animals parasitized on a farm, or by the numbers of worms per fox, the extent of infestation varies widely, and there seems to be an inverse correlation between infestation with this parasite and infestation with the other lungworm, *Crenosoma vulpis*. The evidence for this correlation will be discussed in the next section.

The figures obtained from the cubs do not give an adequate picture of the distribution of the worms over the various farms, because of the comparatively small numbers of carcasses examined, and also because only a small proportion of those sent in could on our findings be expected to carry such species as *Crenosoma vulpis*, *Eucoleus aerophilus*, and *Capillaria plica*. We have only found these three worms in carcasses of comparatively late cubs, and as a large proportion of the animals we received were much younger than the earliest age at which any of the above three species have been found it follows that they cannot be used to assess the status of these worms. On the other hand, the findings for *Toxocara canis* and *Uncinaria stenocephala*, both of which appear at a comparatively early age, present a much more complete and continuous picture, which corresponds roughly with that presented by the figures for the adult foxes.

The existence of an inverse correlation between infestations with Crenosoma vulpis *and with* Eucoleus aerophilus

From Table 5 and Fig. 1 it seems possible that there may exist an inverse correlation between the infestations with *Crenosoma vulpis* and those with *Eucoleus aerophilus*. On farms 1, 2, 5, 8, and 9, where *Crenosoma* is comparatively scarce, the proportion of foxes examined which carried *Eucoleus* ranges between 76·9 and 100%, and is uniformly higher than it is on the other farms, where *Crenosoma* is common. Farm 3 provides an exception, a high percentage of the foxes examined being infested with both lungworms. If the farms concerned are treated in two groups it is obvious that on those farms where *C. vulpis* is scarce not only is a larger proportion of the foxes infested with *Eucoleus* but also the average number of worms in each fox is very much higher than on the farms infested with *Crenosoma*. Table 7 shows that on each farm concerned the average number of *Eucoleus* is higher in those foxes carrying it alone of the lungworms, than in those which carry both it and *Crenosoma*, while the averages over all foxes examined are 28·5 in foxes carrying *Eucoleus* alone, as against 21·5 in foxes carrying both parasites.

It is not possible to draw a satisfactory conclusion as to the opposite relationship, as only twelve foxes have been found to carry *Crenosoma* alone. It will be seen that on two farms the foxes with this species alone carry a lower average number than those with both worms, while on the most heavily infested farm the opposite is the case. The high figures from this farm so weight the totals that the averages for all foxes examined give 37·4 *Crenosoma* in each of those foxes which also carry *Eucoleus*, as opposed to 54·2 in those in which *Crenosoma* alone was found. As, however, the ratio of *Crenosoma* to

[1] During sexing of this species by one of us (C. V. W.), the male spicules and sheath characteristic of *Capillaria* were observed. This has been confirmed in a private communication by Dr H. A. Baylis, but we have decided to retain the name *Eucoleus aerophilus* in place of *Capillaria aerophila* throughout this article to avoid any possibility of confusion with *C. plica*.

Eucoleus in those animals which carry both is 1·76 to 1, or nearly 2 to 1, it may be legitimate to suppose that *Crenosoma* will have a more marked effect on *Eucoleus* than the reverse. The severe broncho-pneumonia typical of *Crenosoma* infestation may also be favourable to this species and the reverse for *Eucoleus*.

Table 7. *Relations between infestations with* Eucoleus aerophilus *and* Crenosoma vulpis

Farm	No. of foxes	No. of foxes with *E. aerophilus* alone	No. of foxes with *C. vulpis* alone	No. of foxes with *E. aerophilus* and *C. vulpis*	Average *E. aerophilus* per fox	Average *C. vulpis* per fox	Average *E. aerophilus* per fox with *C. vulpis* also	Average *E. aerophilus* per fox with *E. aerophilus* alone	Average *C. vulpis* per fox with *E. aerophilus* also	Average *C. vulpis* per fox with *C. vulpis* alone
2	18	10	0	7	26·4	9·3	19·7	33·5	22·4	—
5	13	7	0	4	15·5	3·8	3·2	27·1	12·2	—
1	9	8	0	0	36·9	0	—	41·5	0	—
8	6	3	0	3	5·8	2·2	5·7	6·0	4·3	—
3	20	2	0	15	35·7	15·5	41·9	43·5	20·7	—
4	11	0	4	6	4·7	43·2	8·7	—	58·0	31·7
7	9	0	5	4	2·1	63·8	4·7	—	95·2	18·6
6	7	0	3	4	7·1	111·1	12·5	—	87·0	143·3
9	5	4	0	0	1·6	—	—	2·0	—	—
Total	98	34	12	43	19·3	23·1	21·4	28·5	37·6	54·2

Table 8. *Average infestations in age and health groups*

Notes. (i) = average number of worms per fox. (ii) = larva or egg/female ratio.

Age group	No. of foxes	*C. vulpis* i	*C. vulpis* ii	*E. aerophilus* i	*E. aerophilus* ii	*T. canis* i	*T. canis* ii	*U. stenocephala* i	*U. stenocephala* ii	*C. plica* i
2 yr. or more	9	1·7	0	3	368	2·9	283	5·9	100	12
Healthy	8	2·0	0	3	368	3·5	283	7	100	13
Unhealthy	—	—	—	—	—	—	—	—	—	—
Health unknown	1	0	0	0	0	0	0	0	0	0
1–2 yr.	11	4	5	4	65	1	514	26	29	18
Healthy	5	5	7	6	37	2	457	16	17	16
Unhealthy	3	5	0	4	133	0	0	29	104	9
Health unknown	3	0	0	1	100	0	0	39	14	31
7–9 m.	40	42	17	19	14	13	61	50	39	23
Healthy	21	23	13	13	211	18	103	40	43	23
Unhealthy	14	76	17	31	111	10	94	50	50	22
Health unknown	5	31	28	8	11	1	283	94	26	28
84 d.–5 m.	16	0·3	33	120+	23	14	44	53	27	2
57–83 d.	5	0	0	0	0	6	167	30	56	0
29–56 d.	18	0·1	0	3	59	34	1242	103	158	0
7–28 d.	5	0	0	0	0	6	0	0	0	0
0–6 d.	8	0	0	0	0	0	0	0	0	0

The ages at which infestations first occur

In Table 8 are listed the average figures for the age and health groups of the foxes. It is apparent that *Toxocara* is the first to appear, at an age of between 7 and 28 days. The actual records (see Table 4) are: no. 128, age 1–2 weeks, with six immature worms; no. 126, age 3 weeks, with one immature worm; and no. 83, age 18 days, with six immature worms. The earliest time of appearance noted is therefore between 7 and 14 days. No mature, ovipositing worms have, however, been recorded before an age of 28–29 days (foxes 12 and 129). *Uncinaria* has first been recorded at 46 days (fox 9, a single specimen), and by about 2 months may be very numerous (foxes 96, 98, 101, 102). *Crenosoma*,

Eucoleus, and *Capillaria* all appear at approximately the same comparatively late age, about 2½ months. Foxes 96 and 103 afford an indication, however, that the two lungworms may under certain circumstances appear at an earlier date, somewhere between 4 and 8 weeks. In the case of *Eucoleus* cubs 9, aged 46 days, and 71, aged 1–2 months, provide extraordinary instances of relatively high egg counts from the faeces in the apparent absence of worms in the carcass. It is difficult to account for this. We have not infrequently observed the same phenomenon in the adult foxes, and have attributed it to the recent expulsion of ovipositing females. It is possible, however, in these cases that the females are actually present in the nasal passage which was not usually examined (see Hanson & McBlain, 1935). It is unlikely, however, that such expulsion or migration can have already occurred in such young animals, and the only possible explanation that can be offered is that the cubs were running on ground thickly strewn with the eggs, and that the eggs found in the faeces have been picked up and passed through the gut unaltered.

From the point of view of the life cycle of the fox the nematode parasites fall into two groups: *Toxocara canis* and *Uncinaria stenocephala*, which appear before or at weaning; and the two lungworms and *Capillaria plica* which usually first appear at about a fortnight after weaning. This affords support to the view that the two worms comprising the first group may infest prenatally, through the maternal blood stream and the placenta. It also affords some indication that there is no likelihood of prenatal infection with the three worms of the second group.

Resistance to infection with nematode parasites

Much work has been carried out, principally in America, on the problem of the immunity exhibited or developed by various hosts to nematode parasites (Herrick, 1926, 1928; Africa, 1931; Chandler, 1932, 1935; Winfield, 1933; Taylor, 1934; Foster, 1935). The results obtained vary considerably, but there is little doubt that some animals show a definite age immunity to certain worms, and that under certain circumstances an immunity may be acquired as a result of previous infestation. The nature of our observations does not permit us to do more than suggest possibilities. In Table 8, in which our results have been summarized under age groups, there are indications that immunity, however attained, may become quite marked. Unfortunately, the number of animals in each age group varies within wide limits, and is in no case very large. It will be seen, however, that in the cases of all the five nematodes with which the foxes are commonly infested, after the time of first infestation, there is a period of comparatively rapid increase in average numbers. Following this the averages gradually drop until, in those foxes of 2 years of age and more, the figures are very low. The average infestations have been plotted in Fig. 2. The marked drop in numbers in the age group 57–83 days is not regarded as significant in view of the small number of animals examined between these ages. In each case, if the figures for the 57–83 days' animals be ignored, the number of worms found reaches a peak soon after the age at which the species has first been observed, and then falls rapidly away. It may be argued that the fall in numbers may be due to selective elimination by the farmers of those heavily infested animals which are thereby of little use for the purpose of either pelt or stud, and which are, moreover, a source of danger to the other animals on the farm. To some extent this may be true of *Uncinaria* and *Eucoleus*, both of which show their presence by the effects which they

produce in the health of the foxes they infest. On the other hand, no owner in the area has claimed to be able to detect either *Crenosoma* or *Capillaria plica* in the foxes. *C. plica*, in particular, does not appear to show its presence in the behaviour or appearance of its host, apart from occasional trouble experienced in micturation, and suspected difficulty in mating. The conclusive argument against selective elimination, however, is that the observed averages have all reached their peak before or at the time when selection begins

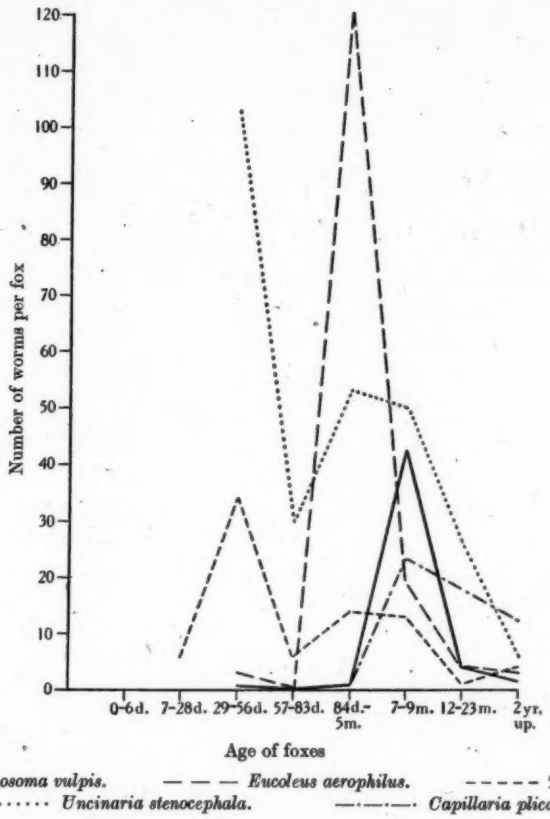

Fig. 2. Age variations in infestations. Averages all foxes.

to operate; that is at the 7–9 months' period when the cubs to be kept for breeding are retained, and the others pelted. There is therefore little doubt that by the age of about 5 months or a little later in some cases, most foxes have developed a marked resistance to the common nematode parasites which infest them. As to whether this resistance is inherent or is acquired we cannot say, the nature of our observations providing no evidence. Hanson & McBlain (1935) believe, however, that in the case of *Eucoleus aerophilus* at least, there is a definite acquisition of immunity, and their observations, although limited to a small number of animals, support this view.

The relation between worm egg or larva counts in the faeces and the number of worms in the carcass

As has been indicated, in the course of the autopsy of each carcass the numbers of worm eggs or larvae in the faeces in the rectum were estimated by the Stoll dilution count. The initial object of this procedure was to attempt to establish criteria for quantitative estimations, however rough, of infestations in living foxes, by the examination of their droppings. The results obtained varied so erratically and over so wide a range that unfortunately it has been quite impossible to establish any criteria whatever on the basis

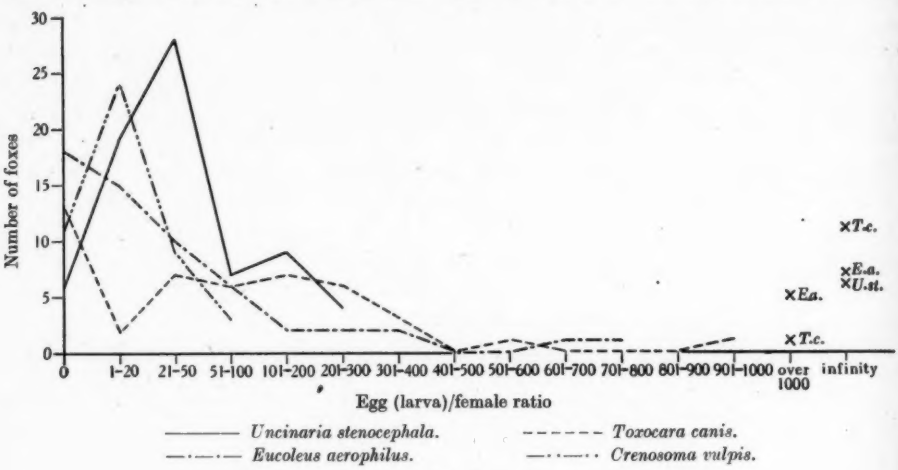

Fig. 3.

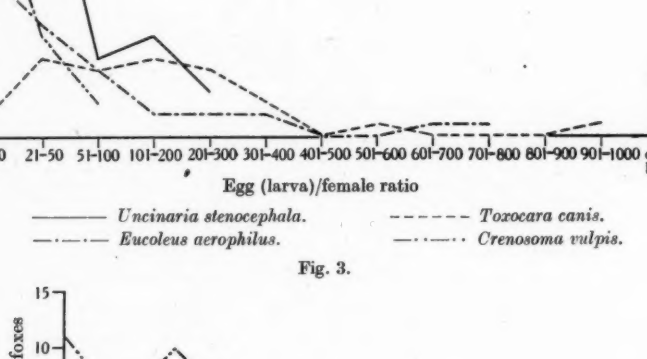

Fig. 4.

of eggs per gram from single faeces samples. This variation is particularly noticeable in the eggs of *Toxocara* and of *Eucoleus*, as may be seen from the curves plotted in Fig. 3. In the majority of cases the numbers of eggs per gram per female worm of either of these species is comparatively low, but very high ratios are found with sufficient frequency to vitiate any possibility of predicting the worm population within any reasonable limits. The curves obtained for both *Crenosoma* and *Uncinaria* correspond much more closely to normal distribution curves with a definite maximum, at 1–20 larvae per gram per female for *Crenosoma*, and at 21–50 eggs per gram per female for *Uncinaria*. Closer analysis of the figures in the neighbourhoods of these two maxima is given in Fig. 4, from which it may be seen that the *Crenosoma* larvae present a definite maximum at

between 6 and 10 per gram per female, whereas the egg production per female *Uncinaria* ranges widely between 6 and 40 per gram per female.

It is therefore established that the larva/female ratio of *Crenosoma* is relatively constant in the samples examined, while the egg/female ratio of *Uncinaria* varies over a comparatively narrow range, and those of *Eucoleus* and *Toxocara* are markedly inconstant. Some significance may be found in the fact that the most constant ratio is provided by the only viviparous species. After leaving the female parent the young forms have travelled a greater or less distance through the gut of the host before being estimated in the faeces sample. A variety of influences during this passage may affect the concentration of the eggs or larvae in the faeces at any one time. All the young forms will be influenced by the movements of the contents of the gut, which will vary according (i) to the times at which the eggs are laid, or passed into the gut, relative to the times of meals of the host, (ii) to the time of day, and so to the activity or sleeping periods of the fox, and (iii) to the condition of the animal, whether constipated, normal, or loose. Very little information exists as to the effects of these various factors on the distribution of eggs in the faeces. As to the last factor, it has generally been assumed that constipation will lead to a high concentration of eggs per gram of sample, owing to the high concentration of faecal matter relative to water. Observations which appear to contradict this view will be given later. In the case of the lung worms further complication is introduced by the necessity of the passage of eggs or larvae from their point of production in the trachea or lungs into the gut. It is not known whether active migration of *Crenosoma* larvae occurs, or to what extent eggs or larvae of the two lung worms are lost by being coughed out, or side-tracked into the nasal cavity. Moreover, the spasmodic nature of the cough reaction by means of which it may be presumed most of the young forms are transported into the alimentary tract, will undoubtedly have the effect of zoning them in the gut, with the result that the faeces of the same animal will at one time show a high count, and at another a much lower one. Under these conditions the markedly more uniform counts obtained for the *Crenosoma* larvae may be due to their motility, and hence the likelihood of their becoming more evenly distributed throughout the contents of the gut. On the other hand, the high variability in the concentration of the eggs of *Eucoleus* may be correlated with the fact that these eggs are laid in adherent strings in the trachea, and are, moreover, generally embedded in the mucus which is produced in quantity by the tracheal mucosa in response to the irritation by the parent worms. It is possible, and even probable, that these eggs tend to remain in close clumps throughout their passage through the gut, with consequent marked variability in the faecal egg-counts. The differences between *Uncinaria* and *Toxocara* may be partially accounted for by two facts: (i) *Toxocara* is the most easily ejected of the common nematodes infesting silver foxes, and routine pilling is carried out against it. The populations of this worm are therefore liable to sudden and artificial changes, and it is possible that even if some worms are not killed their reproductive rate may be adversely affected by the vermicide. *Uncinaria* is much more resistant to vermicides and is therefore not liable to disturbance to anything like the same degree. (ii) Whereas the egg of *Uncinaria* is thin-shelled, of smooth surface, and of comparatively streamlined shape, that of *Toxocara* is comparatively large, thick-shelled, spherical and of roughened surface. The egg of *Uncinaria* is therefore more likely to become evenly distributed in the gut contents, and ultimately in the faeces, than that of *Toxocara*.

In view of the erratic nature of the results just outlined it was decided to obtain faeces samples at intervals before killing from a selected group of foxes, and to correlate the counts obtained from the droppings with those from the faeces in the rectum of the carcass, and both counts with the worm population at death. With the co-operation of the owners of six farms, droppings were sent in from a large number of animals which were destined for pelting. From these altogether twenty-seven animals were selected, representing a cross-section of the known conditions as far as possible. In order to minimize the risk of actual changes in the worm populations over a long period of time, most of these animals were studied for only about 1 month before being pelted. During that period between two and five faeces samples were sent in and examined, and in some cases the last sample was taken immediately before the animal was pelted. In the final event only twenty-five of the foxes could be used, since two of them proved to have no faeces in the rectum on being autopsied. These could not therefore be used for comparative purposes. Space does not permit the full results to be tabulated here, but two cases are given in Table 9 as examples typifying the extreme nature of the fluctuations

Table 9. *Comparison of rectal with droppings counts, and of both with the worm population in the carcass*

Notes. Larvae or eggs indicates the worm larva or egg count per gram of faeces. Figures in brackets indicate numbers of mature females.

		C. vulpis		*E. aerophilus*		*T. canis*		*U. stenocephala*	
	Date	Worms	Larvae	Worms	Eggs	Worms	Eggs	Worms	Eggs
Fox 116:									
Carcass	29 Dec.	84 (41)	166	1 (0)	2766	71 (26)	4766	0	33
Droppings	20 Oct.		1166		533		533		66
	2 Nov.		466		1100		3500		66
	19 Nov.		0		866		666		0
	14 Dec.		300		3166		1366		133
Fox 153:									
Carcass	24 Feb.	11 (6)	166	49 (20)	733	5 (2)	0	11 (7)	966
Droppings	8 Feb.		0		66		0		666
	11 Feb.		133		100		0		566
	15 Feb.		233		33		0		33

in the faecal counts at comparatively short intervals. The examples selected represent the type of variation characteristic of the whole series. Three noteworthy facts emerge: (*a*) the high degree and erratic nature of variation exhibited by the egg or larva counts in the droppings, at comparatively short intervals; (*b*) the marked lack of correlation between the droppings counts and the rectal counts; (*c*) the absence of any critical relationship between the numbers of worms present in the carcass at autopsy and the egg or larva counts, whether from the droppings or from the rectal contents. It must be admitted that in the more extreme cases 2 months have elapsed between the time of examination of the first droppings sample and that at which the autopsy was performed, and that during this time quite considerable changes may have taken place in the worm populations within the animal. But the figures we have obtained rarely show progressive increase or decrease in worm eggs or larvae, as might be expected if the worm infestation were in course of change. It is interesting to note that in no single case where eggs were present was uniformity observed between droppings and carcass counts. The absence of any constant ratio between droppings counts and rectal counts is shown in that for each species of worm the cases of excess of carcass count over droppings count are approximately equalled by those of deficit.

Having failed by these means to establish any diagnostic criteria from single faeces counts, or from interrupted series of counts, it was decided to estimate the total egg production per day from individual foxes over a period of several days. At the kind invitation of Mr C. J. Johnstone of Dartmoor Fur Farms, Ltd., Chagford, one of us (L. A. H.) spent some 10 days at his farm and was able to study several foxes. Owing to the organization of the farm it was not possible within a reasonable period of time to carry out autopsies on the animals selected, as none of the animals due for pelting are run

Table 10. *Rates of egg production by* Uncinaria stenocephala *and* Eucoleus aerophilus

Period hr.	Faeces g.	Faeces per hr. g.	*U. stenocephala* Eggs per g.	*U. stenocephala* Total eggs	*U. stenocephala* Eggs per hr.	*E. aerophilus* Eggs per g.	*E. aerophilus* Total eggs	*E. aerophilus* Eggs per hr.	Remarks
Eva, vixen									
1. 9	30	3·3	33	990	110	66	1980	220	
2. 15	50	3·3	33	1,650	110	133	6650	433	
3. 9	65	7·2	66	4,290	477	33	2145	238	
4. 15	60	4·0	0	0	0	0	0	0	
5. 9	45	5·0	66	2,970	330	0	0	0	
6. 15	20	1·3	100	2,000	133	0	0	0	
7. 9	35	3·9	0	0	0	66	2310	257	
8. 15	20	1·3	0	0	0	33	660	44	
9. 9	80	8·9	33	2,640	293	66	5280	587	
10. 15	20	1·3	0	0	0	33	660	44	
11. 9	60	6·7	0	0	0	33	1980	220	
12. 15	30	2·0	0	0	0	33	990	66	
13. 9	45	5·0	0	0	0	0	0	0	
14. 15	25	1·7	0	0	0	0	0	0	
Radiant, vixen									
1. 15	75	5·0	166	12,450	830				
2. 9	35	3·9	600	21,000	2333				
3. 15	50	3·3	700	35,000	2333				
4. 9	60	6·7	400	24,000	2667				
5. 15	75	5·0	333	24,975	1665				
Alice, vixen									
1. 9	70	7·8	100	7,000	778				
2. 15	70	4·7	33	2,310	154				
3. 9	20	2·2	166	3,320	367				
4. 15	30	2·0	366	10,980	732				
Fantasy, vixen									
1. 15	60	4·0	366	21,960	1464				
2. 9	45	5·0	633	28,485	3165				
3. 15	20	1·3	366	7,360	491				
4. 9	15	1·7	33	495	55	—	—	—	Constipated
5. 15	55	3·7	266	14,630	975				
6. 9	10	1·1	266	2,660	295	—	—	—	Constipated
7. 15	55	3·7	600	33,000	2200				
8. 9	15	1·7	333	4,995	555	—	—	—	Constipated
9. 15	60	4·0	100	6,000	400				

separately. The figures can therefore only be related to one another. In all, four foxes were studied over greater or less periods. Three of them were passing only eggs of *Uncinaria*, and these were followed for from 48–60 hr. each. The other was passing eggs of both *Uncinaria* and *Eucoleus*, and this animal was followed for 7 days. Owing to lack of time it was decided to use composite samples over 12 hr. periods. The pens were carefully gone over every 12 hr. and all the droppings from each selected fox brought in. They were first weighed, and then after thorough stirring and mixing, the Stoll dilution counts were made. This procedure gave the average number of eggs per gram passed during the 12 hr. period. The results obtained have been detailed in Table 10, from

which it may be seen that, whether the egg production be estimated against the bulk of faeces passed, or against time, there appears no constancy in the rate of their production. This applies equally for both *Eucoleus* and *Uncinaria*. It must therefore be concluded that for the common parasites of the silver fox the numbers of eggs or larvae found in the droppings vary so greatly and with such irregularity that they afford no reliable indication of the degree to which the animals are infested with the worms by which they have been produced.

Various attempts have been made to show that the concentration of worm eggs in the faeces may vary according to the hardness or softness of the latter. There is no doubt that in the fox constipation has a marked effect on the worm egg count, as may be seen by reference to the tabulated results just given. It will be noted, however, that the effect is by no means uniform in its direction or intensity. In some cases the number of eggs per gram is higher than normal, in other cases lower, although in every case the total number of eggs present in the sample is low, owing to the markedly smaller weight of the droppings passed during these periods.

It has already been mentioned that anomalous cases may occur of eggs being found in the faeces in the absence of any adult worms in the body, and a possible explanation of such occurrences of the eggs of *Eucoleus* in young cubs has been suggested. The distribution of the cases recorded among the different species of worms is significant. Ten cases (three being in cubs) are recorded of *Eucoleus* eggs, seven (one cub) of *Toxocara*, one of *Uncinaria* (only one egg being found in three counts), and none of *Crenosoma*. The only two species which give a relatively high proportion of such occurrences are those which are most likely to be expelled suddenly, the one by being coughed out, the other by vermicide.

Table 11. *The sex ratios of the worms*

Note. Figures in brackets indicate percentage of total number of cases for each species.

			T. canis			
% female	*C. vulpis*	*E. aerophilus*	Adult	Immature	*U. stenocephala*	*C. plica*
0– 10	2 (3·4)	9 (11·0)	9 (11·0)	16 (10)	2 (1·8)	—
11– 20	—	1 (1·2)	5 (6·1)	3	—	1 (1·7)
21– 30	—	2 (2·4)	5 (6·1)	2	—	1 (1·7)
31– 40	3 (5·1)	5 (6·1)	15 (18·3)	5	8 (7·3)	2 (3·4)
41– 50	11 (18·6)	17 (20·7)	16 (19·5)	8	15 (13·8)	7 (11·9)
51– 60	7 (11·9)	12 (14·6)	8 (9·8)	5	29 (26·6)	18 (30·5)
61– 70	15 (25·4)	12 (14·6)	9 (11·0)	3	27 (24·8)	12 (20·3)
71– 80	6 (10·2)	4 (4·9)	5 (6·1)	—	16 (14·7)	7 (11·9)
81– 90	2 (3·4)	—	—	—	1 (0·9)	1 (1·7)
91–100	13 (22·0)	20 (24·4)	10 (12·2)	14	9 (8·3)	6 (10·2)
% female in total	61·9	55·6	49·3	47·9	58·2	62·2

The sex ratios of the worms

Owing to press of work at times, it was not always possible to sex the worms removed at autopsy. They were, however, sexed for all the five species found in over one hundred foxes, and in Table 11 the results are expressed in terms of the percentage of females among the population of each species of worm in the foxes. Over the total numbers in all foxes, for each species, with one exception, the females are in marked excess over the males, the percentage ranging from 55·5 to 62·0. The exception is provided by *Toxocara canis*, where males are in slight excess, 47% of the total being female. It is perhaps significant that once again the exception is provided by the species which suc-

cumbs most readily to vermicides. There is some evidence for the belief that the male is less susceptible than the female to vermicides in several species of nematode, and also that he is the longer lived, owing presumably to the heavy strain which is imposed on the female by egg production.

The distribution of the male and female worms in the foxes has been plotted in Fig. 5. For comparative purposes the numbers of occurrences of each ratio have been reduced to percentages of the total number of foxes in which each species has been sexed. A striking fact evident is that in the case of each of the five species of worm the curve shows a sharp rise at the point 90–100% female. Actually, in every case, the ratio is 100% female, no males of the species being present in the carcass. Some of these animals contained a single worm only, in which case the percentage of the sex it represents must of course be one hundred. These cases have therefore been separated from those in which more than one worm has been found, but in which nevertheless only one sex has been represented. In the figure, the continuous line represents the percentage of all animals carrying 100% of either sex, while the dotted line indicates the percentage of animals carrying more than one of the species, but these of one sex only. In all, on no less than sixty occasions female worms alone have been present, as against twenty-one occurrences of males alone. *Toxocara* alone shows an excess of males alone over those of females alone, when more than one of the species has been found. Even so the large number of cases of single females of this species so weights the final figures that there is a slight excess of cases of 100% female over those of 0%. Since the ratio of females to males barely exceeds 3 : 2 at its highest, and in the case of *Toxocara* is less than 1 : 1, the discrepancies between the two sets of figures cannot be accounted for on the basis of random variation. It is reasonable, however, to suppose that random variation will modify whatever factor is operating, and this is borne out by the figures for *Toxocara*. Apart from this, however, little light can be thrown on the observations, because nothing is known of the history of any of the infestations. It is suggestive that the highest proportion of cases of 100% female occurs in the two lungworms, neither of which is normally expelled by vermicide. On the other hand, if one considers the ratios of the sexes among immature forms, irrespective of whether they are accompanied by adults or no, the few occurrences among our foxes of immature forms of *Crenosoma*, *Eucoleus*, and *Uncinaria* are all 100% female, except for one fox which carried 100% male *Eucoleus*. The numbers of cases recorded for each genus are: *Crenosoma* 2 (1), *Eucoleus* 1 (0), *Uncinaria* 3 (2). (The figures in brackets indicate cases of the occurrence of single immature worms.) *Toxocara* alone was found freely in immature form, and the distribution of the sex ratios of these among the foxes examined is stated in a separate column in Table 11. The total numbers of immature *Toxocara* are 175 male to 161 female, i.e. 48% female. This ratio, and the distribution of ratios among the foxes, compare very closely with those shown by the combined immature forms and adults. This suggests that the balance is established early in the history of the infestation and is not due to differential elimination of the adult or maturing worms.

The parasite population of the British wild red fox, Canis vulpis

It seemed important to know what likelihood there was of infestation of the silver-fox farms by parasites derived ultimately from the wild-fox population of the neighbourhood. While no direct access was possible between these foxes and the domestic population,

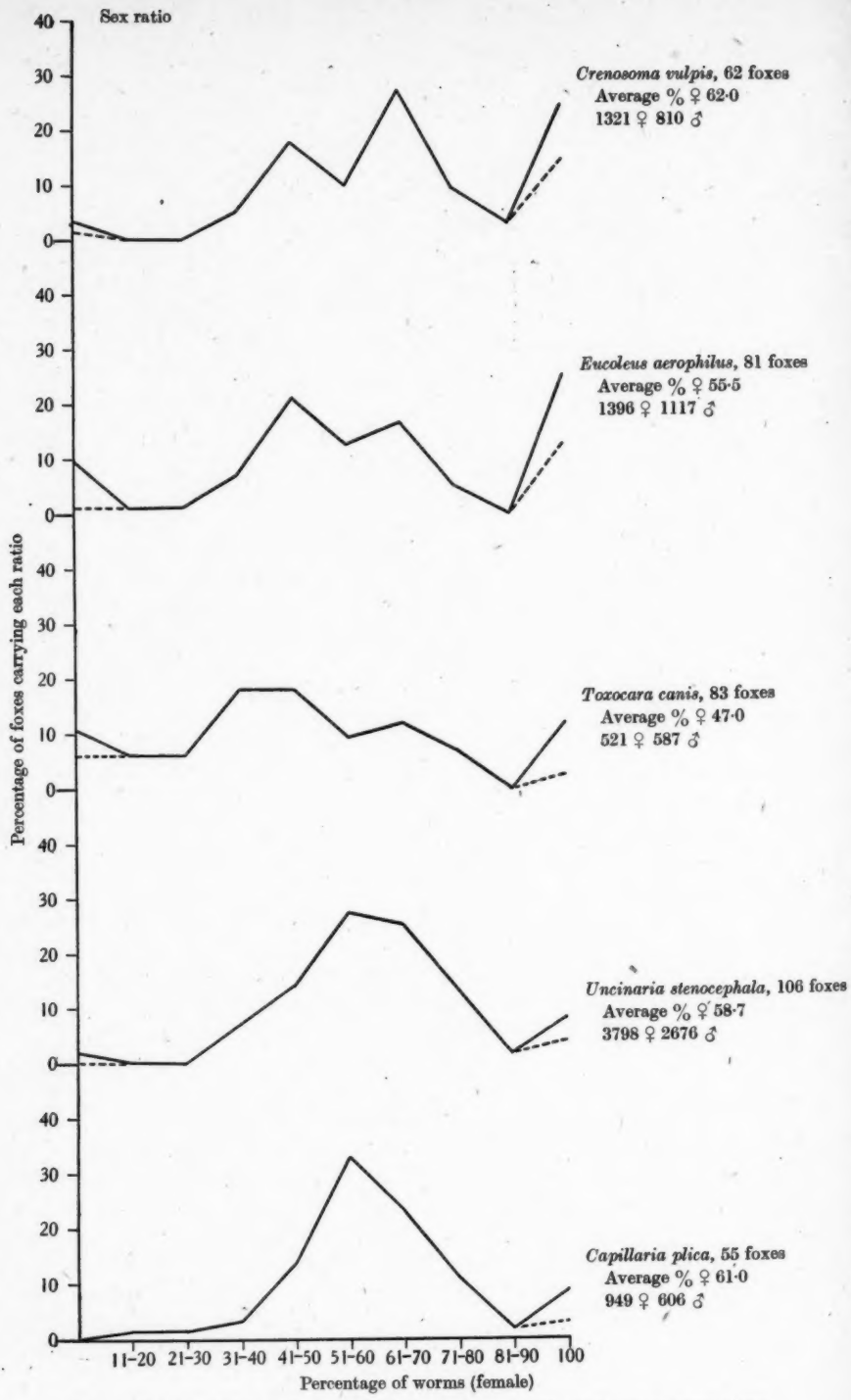

Fig. 5. The dotted lines indicate percentage infestations with single sex of worm where more than one worm is present.

there was, nevertheless, a distinct possibility that parasites might invade the farms by being blown on the wind as eggs, or carried in in mud on the feet of men and animals; or where an intermediate host intervenes in the life cycle as in the case of *Crenosoma vulpis*, by transport in such hosts. The chances of such infestation are increased during the breeding season when red foxes are frequently seen about the outer guard fences, attracted by the breeding calls of the vixens. Also, on the rare occasions when silver foxes have escaped into open country and have later been recaptured, it is highly possible that they may have become infested by direct contact with the wild foxes while at liberty.

An attempt was therefore made to obtain a sample of carcasses of the red fox for examination. This was only partially successful, because, in a hunting country, fox carcasses are not easily come by. We obtained only two carcasses from the south-west, the remaining twelve coming from Oswestry. These, however, provide some indication of the degree to which infestation may be expected. The age of the animals was, of course, unknown, except approximately, in the cases of four cubs.

Table 12. *Distribution of parasites. Wild red foxes*

Notes. As in Table 1. Neither *C. vulpis* nor *E. vulpis* was found.

Fox	Sex	Wt. lb.	Wt. oz.	*E. aerophilus* i	*E. aerophilus* ii	*E. aerophilus* iii	*T. canis* i	*T. canis* ii	*T. canis* iii	*U. stenocephala* i	*U. stenocephala* ii	*U. stenocephala* iii	*T. pisiformis*	*C. plica*
1	m.	15	6	0	0	—	(2)	0	—	11	66	9	11	0
2				4	0	0	(1)	0	—	4	133	44	4	9
3	m.	16	8	2	66	66	(2)	0	—	5	0	0	7	0
4	m.	18	0	2	0	0	4 (2)	100	33	3	0	0	12	16
5	m.	13	0	0	n.f.		9	n.f.		5	n.f.		77	7
6	f.	13	0	2	n.f.		(1)	n.f.		16	n.f.		0	1
7	f.	13	0	4	0	0	(1)	0	—	92	66	1	121	14
8	m.			0	n.f.		0	n.f.		20	n.f.		3	30
9	m.	13	8	0	n.f.		3	n.f.		7	n.f.		0	0
10	f.	14	8	1	n.f.		4	n.f.		66	n.f.		4	3
11, cub	m.	4	1½	0	n.f.		1 (1)	n.f.		4	n.f.		5	0
12, cub	f.	4	2	0	n.f.		0	n.f.		5	n.f.		3	0
13, cub	m.	5	0	3	n.f.		0	n.f.		21	n.f.		5	0
14, cub	f.	5	0	0	n.f.		1 (2)	n.f.		3	n.f.		7	0

The results of our examinations are detailed in Table 12. The following points are noteworthy:

(i) *Crenosoma vulpis* was entirely absent from the sample, and the other lungworm, *Eucoleus aerophilus*, while present in 50% of the carcasses, was invariably represented by one or two individuals only.

(ii) *Toxocara canis* was generally distributed, occurring in eleven of the fourteen foxes. Like *Eucoleus aerophilus*, however, it was rarely represented in large numbers, and of these the remarkably high proportion of 28% were immature. It would seem therefore that the wild fox develops a high degree of resistance to *Toxocara canis* and that the parasite is very readily expelled.

(iii) *Uncinaria stenocephala* was present in all the foxes examined, frequently in large numbers, the average infestation over the whole sample being nineteen worms per fox.

(iv) *Taenia pisiformis* was absent from two only of the carcasses, and in two instances was present in numbers far higher than those found in silver foxes. It seems therefore

that under natural conditions foxes are very susceptible to infestation with this tapeworm, and it is therefore well worth while for the fox farmer to take careful measures to exclude this parasite from the food fed to his foxes.

(v) *Capillaria plica* occurs in 50% of the sample, but usually in moderate numbers, and in no case affecting the kidney.

(vi) *Eimeria vulpis* was not recorded in any of the foxes examined.

(vii) A single specimen of *Linguatula serrata*, an immature male, occurred in the gut of one of the foxes.

As far therefore as the internal parasites are concerned, it seems that the hookworm is the species most to be guarded against. All the other parasites found in silver foxes, except *Eimeria* and *Crenosoma*, also occur with sufficient frequency in wild foxes for there to be a distinct possibility of their transmission to the stock on the farms.

Of external parasites, *Otodectes cynotis* was recorded in one instance only, fleas were found in two of the pelts, proving to be *Spilopsylla cuniculi* (5), and *Monopsyllus sciurorum* (1). The only other parasites to be recorded were one *Ixodes ricinus*, and one egg of the louse *Trichodectes vulpis*. The comparative absence of ectoparasites is important, as these might be expected to be the more readily transmissible to the silver-fox stock, and moreover, when present, may do very extensive damage to the skin and pelt, so causing serious losses. It must be remembered, however, that most of the red-fox carcasses had travelled a considerable distance before they were examined, and there was ample time for such active things as fleas in particular to have left the carcass en route.

It is interesting to note that although a special search was made for *Echinococcus granulosus*, it was not found. The existence therefore of a reservoir of this species in the wild red fox has yet to be established.

The only other wild British mammal to be examined in connexion with this survey was the hedgehog. It had been suggested to us that the *Crenosoma* found in the lungs of this animal might be transmissible to foxes. Three hedgehogs were autopsied, one being obtained locally, and two from Oswestry. *C. striatum* was present in the lungs of all three, the numbers found being 110, 10, and 1 respectively. This species is, however, readily distinguishable from *C. vulpis*, and it is unlikely that its presence would have been overlooked among the *Crenosoma* removed from the lungs of the foxes examined. It may be confidently stated therefore that *C. striatum*, of the hedgehog, was not found in the sample of foxes, whether silver or red, which we have examined.

Summary

1. The carcasses of 154 silver foxes of various ages, from fifteen farms in the south-west, have been examined and their parasites recorded. In addition, those of fourteen wild red foxes have similarly been examined.

2. The incidence of various species of nematode, cestode, and protozoan parasites has been analysed for the foxes, in age groups and in farm groups.

3. The worm egg counts in the faeces have been discussed in relation to the numbers of female worms in the foxes. No adequate correlation has been established between the two sets of data.

4. The ages of the foxes at incidence of the nematode parasites has been discussed, and the possibility of the development of resistance to various species investigated.

5. Certain peculiarities in the sex ratios of the Nematoda have been discussed.

6. The possibility of infestation of silver foxes by parasites derived from the wild red-fox population of the neighbourhood of the farms has been investigated, as has also that of the transference of *Crenosoma striatum* from the hedgehog to foxes.

The authors wish to express their deep gratitude to all those helpers who have made the investigation possible: to E. L. Taylor, Esq., of the Laboratory, Weybridge, for his continued help and advice, for the examination of certain foxes at a time of extreme pressure, and for the identification of specimens sent to him from time to time; to Dr W. A. Robb, M.D., of the Devon and Exeter Hospital, for the investigation of a number of brains of foxes suspected of encephalitis; to the owners of fox farms in the area, who have most readily co-operated with us, supplying carcasses for examination together with details of their histories and of the herds on their farms. Particularly are we obliged to Mr C. J. Johnstone of Dartmoor Fur Farms, Ltd., who has been at all times keenly interested in our work, and who provided facilities for one of us to work on his farm during August 1938. Finally, our thanks are due to Messrs White and Collins, on whom the brunt of the actual examinations has all too frequently fallen.

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NATURAL POPULATION OF THE HEAD-LOUSE (*PEDICULUS HUMANUS CAPITIS: ANOPLURA*) ON INFECTED CHILDREN IN ENGLAND

By KENNETH MELLANBY[1]

From the University of Sheffield

(With 2 Figures in the Text)

Two important figures concerning head-lice in any country are the proportion of people infested, and the number of lice living on the infested individuals. Buxton (1936, 1938, 1940, 1941) has given extensive data from a number of African and Asiatic areas. Earlier workers have only given a few odd records for individuals in England, and no figures from which the average degree of infestation may be deduced.

I have previously (Mellanby, 1941) described the results of an investigation into the frequency of infestation in England; this covered about 60,000 individuals of all ages, examined over a period of several years, from widely different environments. The main conclusions are here summarized in Table 1, which shows that head-lice are very common in industrial cities, where practically half the girls under 14 may be infested. The small boys below school age are almost as lousy as the girls, but the older boys are considerably less affected. In the rural areas head-lice are very much less common.

Table 1. *The percentage infestation with head-lice of individuals of different ages from cities and rural areas. Each number represents the percentage found with lice or nits*

Age ...	Under 1	1–4	5–8	9–13	14–18	19 and over
A. From ten industrial cities (52,445 individuals)						
Males	13	42	33	27	11	1
Females	15	47	49	49	27	9
B. From four rural counties in southern England (2522 individuals)						
Males	Nil	8	5	4	Nil	Nil
Females	Nil	5	8	6	5	Nil

The data presented in this paper give the actual number of living lice found on the heads of children admitted to a hospital in one northern industrial city during the period from November 1939 to the end of October 1940. In the previous report, results from this same city are included under 'County Borough E'.

Methods

Buxton's method for collecting head-lice (Buxton, 1936) in which the heads of a large number of individuals are shaved, the hair dissolved in alkaline sodium sulphide, and the lice, which are insoluble, are then isolated, is unfortunately impracticable in England. The figures given here were obtained as a result of careful combing of the hair of every child admitted into a Children's Hospital. Each child was combed first to see whether or not there was any infestation with *Pediculus*, and when an infestation was found all

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the parasites were combed out and put straight into a jar and sent to me for examination. The two possible sources of error are first that some lice might be overlooked, and secondly that some might be lost before being transferred to the jar. I do not believe that any errors from these causes were substantial, because the whole process was under the close supervision of the Resident Medical Officer. He was constantly on the look-out for possible carelessness on the part of the nursing staff, and ensured that the directions were always properly carried out. It is certain from re-examination that the number of lice missed during the combing was practically nil.

It is very difficult to obtain reliable results from infested adults by this combing method, as the patients are inclined to make difficulties. If, however, an investigation were being conducted personally by a Resident Medical Officer, then difficulties could no doubt be overcome.

At first I hoped to collect all the nits as well as the lice, but this proved impossible. As after combing the heads were so treated that all live nits were killed, the labour of removing them all at one combing, which would have done nothing to improve the health of the patients, was too great for the already busy hospital staff.

RESULTS

During the period under review (November 1939–October 1940), 93 children, 45 boys and 48 girls, were admitted to the hospital with live lice in their hair. It should be noted that their verminous state was never the cause of admission. Of the total admissions over the age of 2 years 28% of the girls and 14% of the boys were lousy. Of these lousy children, live lice were detected in three-fifths of the cases, and in the remainder nits only. I have previously stated that it is unwise from the public health point of view to maintain any distinction between children with live lice and those with nits only, for even if the nits are all dead, live lice must have been breeding in the hair quite recently. It seems likely that when only nits are discovered in a cursory examination a few live lice are actually present. This suggestion is borne out by the fact that prior to November 1939, when this special investigation was begun, two-thirds of the verminous children admitted to this same hospital were recorded as having nits only (as all heads were carefully cleaned of nits, no harm was done if some lice were overlooked). As a result of the careful preliminary examinations made on each child for this investigation, nearly twice the proportion of children was found with live lice.

From all the children 1377 lice were collected, 1180 being larvae, 121 adult males and 76 adult females. The average number of lice per child was 14·8, the figure for boys being 12·7 and for girls 16·8. 79% of the infested children had all stages of lice (adults, larvae and nits) present. As Buxton has previously noted, these arithmetical means have a limited meaning, because they may be unduly weighted by a single large count from one heavily infested head. The most satisfactory method of expressing the data is that adopted by Buxton, in which the proportion of individuals with few lice (1–10 parasites) is compared with the proportion more heavily infested (with 11–100 lice and with 101 and over). This has been done in Figs. 1 and 2. From Fig. 1 it will be seen that in both boys and girls infestations containing under 10 lice are the most common. This figure is strikingly similar to those given by Buxton (1938) for certain African and Asiatic populations; this suggests that factors controlling the head-louse may be similar in widely different parts of the world.

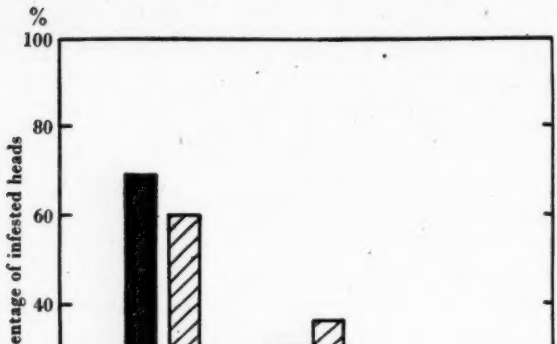

Fig. 1. The number of living head-lice found on infested children. 69% of the boys and 60% of the girls had under 10 lice each.

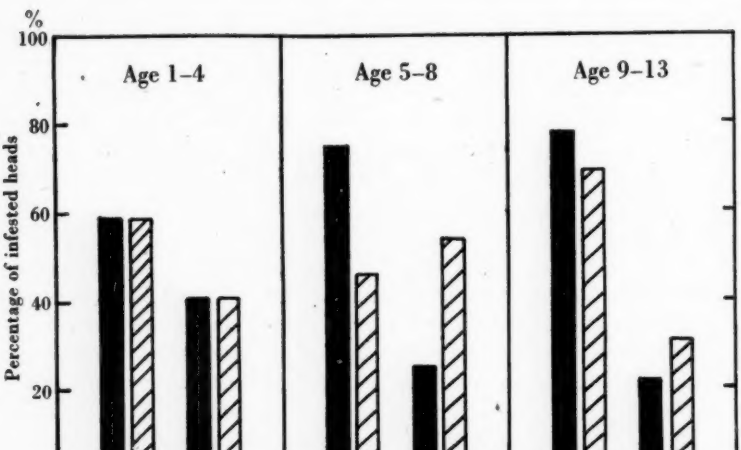

Fig. 2. The number of living lice found on infested children. For each age group the number of boys (black) and girls (striped) with either a light infestation of up to 10 lice, or a heavier infestation of more than 10 is shown.

In Fig. 2 the children are divided into age groups. Among the youngest children, aged 1–4, the degree of infestation is the same in boys and girls. Among the older children, particularly those between 5 and 8, heavy infestation is commoner among girls than boys. This would be expected as the girls have longer hair, and also are more frequently verminous than the boys (see Table 1).

The number of children in each group is rather small, and so the differences commented on in the previous paragraph are not all statistically significant. This does not, however, mean that they may not be real, particularly as certain essentially similar conclusions have been reached by Buxton from large numbers of observations from other countries.

I have previously (Mellanby, 1941) shown that the frequency of infestation may fluctuate, but that there is no clear-cut seasonal incidence in the occurrence of head-lice in England. The figures considered here show no difference in the frequency with which heavy or light infestations occur at different seasons of the year.

DISCUSSION

The majority of infested children (79%) had all stages of lice—adults, larvae and nits—in their hair. From what we know of the duration of each stage in the life cycle (see Buxton, 1939) this must mean that these infestations have been established for some time. If infection takes place by the introduction of a fertilized adult female louse, about 9 days must elapse before her first eggs hatch and any larvae appear. If larvae are responsible for the infection there will be some days' delay before they reach the adult stage, pair and lay eggs. It is probably safe to say that wherever specimens of all stages are found either the infestation has lasted at least a fortnight or there have been several separate infections.

Another reason for knowing that most infestations, even light ones, are fairly long established is derived from the position of the nits. Usually the female lays her eggs on hair very close to the scalp. These nits are fastened firmly, so that even after hatching the empty shell remains in place, and as the hair grows so the egg shell becomes farther from the scalp. Now hair grows at a rate of approximately 0·4 mm. per day, so a nit 3 cm. from the scalp was probably laid 10 weeks previously. If nits are found at intermediate distances, and live lice are also present, it is probable that the infestation has lasted at least for that period.

These long-established light infestations must be kept down by the efforts of the host. It is generally assumed that only very verminous heads, from which parasites will migrate due to overcrowding, are likely to spread the infestation. I believe, however, that these children with only a few lice may also be important spreaders of infestation. When they comb their hair they must frequently remove a few lice, hence the smallness of the population, and it is unlikely that the lice are killed (or even noticed) when they are removed. Lice may live several days away from the host, and these may remain on the combs or in other places to infect members of the same family or school.

None of the heads considered in this paper was so verminous as to suggest that it had been completely neglected for a long period. Nuttall (1917) describes a woman with 1004 live head-lice. I have received part of the hair from a girl of 18 and recovered over a thousand lice—from the description received of the patient I believe that probably there were twice as many parasites. I have frequently heard descriptions of otherwise

clean and smart young women with elaborate coiffures which are never disturbed for weeks and which become vastly infested with lice. Unfortunately, I have no data as to the populations present, but it may well be that present female styles of hairdressing will tend to encourage larger louse populations. It should be remembered that with a little extra neglect (or less disturbance) a small population of lice, such as may be found so commonly, will soon increase to much greater numbers. Buxton has shown that in those African communities where the highest proportion of the people are lousy, the louse populations per head are largest also. It is possible that in some other cities, as 'A' of the previous survey (Mellanby, 1941) where lice are considerably commoner than in 'E', heavier infestations may be more numerous.

The majority of individuals infested with head- or body-lice, in widely separated parts of the world, appears to tolerate very small numbers of parasites; only a small minority supports really large populations of *Pediculus*. It is interesting to note the numerical similarity of my results for English children to Buxton's (1936, 1938, 1940, 1941) figures for non-European countries, and to MacLeod & Craufurd-Benson's (1941) data for body-lice on men in England.

Summary

The louse populations of the heads of 93 infested children, 45 boys and 48 girls under 14 years of age from a northern English industrial city have been examined.

The average number of lice per head was 14·8 for all children, 12·7 for boys and 16·8 for girls. The maximum population observed was 142.

The majority of infestations (65%) were light with under 10 lice. Girls were more frequently heavily infested than boys.

Most of the infestations, though light, must have been established at least for some weeks.

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NEW ECTOPARASITIC MITES (ACARINA) FROM UGANDA

By CHARLES D. RADFORD[1]

(With 19 Figures in the Text)

Introduction

In this paper two new genera of Laelaptidae are raised, the first for four large mites taken on a banded mongoose, the second to fit a number of females taken on a squirrel. In addition to these new genera, two new species of *Liponyssus* and a new species of *Haemolaelaps* are described. All these specimens have been collected in Uganda.

The representatives of the new genus from the banded mongoose (*Mungos mungo macrurus* Thomas) are large specimens, ♂ 1·0 × 0·7 mm., ♀ 1·2 × 0·8 mm., in which the chief character is the presence of a large stigmal plate ventrally on the lateral edge of the body, lying between coxae iii and iv, and the broken or notched outline of the ventral shields in both sexes.

This stigmal plate closely resembles the stigmal plate found in the Ixodidae. Dorsal shield of male entire, female dorsum possessing two shields.

For this genus I propose the name *Mungosicola* with *M. ugandae* as the type species.

The second new genus is raised for a number of females taken on a squirrel (*Protoxerus stangeri centricola* Thomas) in which the genito-ventral and anal shields are fused together, forming a large bulb-like shield, possessing only one pair of spines (the genital) in addition to the three anal spines.

Closely similar to the genus *Ischnolaelaps* Fonseca, 1935, in possessing only the genital pair of spines on the genito-ventral shield, but differing from that genus in having the shield expanded behind coxa iv, and by being united to the large anal shield, this united genito-ventri-anal shield somewhat resembles the genito-ventral and anal shields of the genus *Macrolaelaps* Ewing, 1929, but does not possess the additional three pairs of spines present in the latter genus.

For this genus I propose the name *Ugandolaelaps*, with *U. protoxera* as the type species.

Liponyssus sangsteri n.sp. was found in association with *Ugandolaelaps protoxera* and *Liponyssus liberiensis* Hirst on a squirrel (*Protoxerus stangeri centricola* Thomas), and is named after the collector, Mr R. G. Sangster.

Liponyssus otomys n.sp. was collected on a water rat (*Otomys* sp.).

To Mr G. H. E. Hopkins, Senior Entomologist (Medical) of Kampala, Uganda, I wish to express my indebtedness for supplying the specimens used in the preparation of this paper.

The holotype and allotype slides are being deposited in the British Museum (Natural History) with paratypes in the Radford collection.

Genus **Mungosicola** n.gen.

Large mites, ♀ 1·2 × 0·8 mm., ♂ 1·0 × 0·7 mm.

The most outstanding features of this new genus are the presence of a large stigmal plate between coxae iii and iv, and the absence of a peritreme.

[1] Work carried out with the aid of a Government Grant.

The dorsal surface of femur iii in the male possesses two large comb-like spurs, and in the female one stout comb-like spur and a more slender spur.

Dorsal shield of male entire, female dorsum possessing two shields. The male venter has a sterno-genital shield and a ventri-anal shield with very irregular (notched) outline. The sternal shield of the female almost covers the area between coxae i and iii. The genito-ventral shield is represented by some six small platelets, with the anal shield normal.

The genus is raised to include the type species *Mungosicola ugandae*, found on a banded mongoose (*Mungos mungo macrurus* Thomas). Material, 2 ♂, 2 ♀.

Mungosicola ugandae n.sp.

On the venter of the female (Fig. 1) is shown the sternal shield which extends between coxae i and iii. The anterior margin is straight, antero-lateral corners rounded, lateral border with two concavities, converging posteriorly to a long medial projection which has four small notches on each side. Just anterior to this projection there is a small hole in the shield. The shield bears the usual three pairs of spines and two pairs of slits or pores, placed respectively behind the first and second pairs of spines.

Level with the posterior border of coxa iv there are four small platelets. Another pair of small platelets lies posterior to and flanking these four platelets. The anal shield is broadly pear-shaped, and is situated at some distance from the posterior end of the body.

Between coxae iii and iv at the lateral edge of the body the stigmal plates are very noticeable, but no peritreme is present. In shape they closely resemble the stigmal plate present in the ticks (Ixodidae) (Fig. 2).

Three pairs of spines are placed between the four small platelets, followed by a pair of spines close to the medial line of the body. There are a number of spines flanking this fourth pair of ventral spines, three on the left and five on the right. Flanking the anal shield there is a spine on each side level with the unpaired anal spine.

The anal pore lies in the anterior half of the pear-shaped anal shield, flanked by the paired anal spines, and the unpaired anal spine lies midway between the anal pore and the posterior end of the shield.

Posterior to coxa iv the integument of the venter bears a weakly chitinized area (represented by a dotted line in Fig. 1) which is presumed to be the remnants of the ventri-anal shield.

On the anterior border of coxa ii there is a tooth-like spur. Legs i and iv longer than legs ii and iii. Leg ii stouter than the remainder. Chaetotaxy of legs normal on the ventral surface.

The dorsal surface of the female (Fig. 3) is almost completely covered by two shields, the anterior shield extending to a level with the anterior border of coxa iv. Eight pairs of spines are placed on or close to the edge of the shield.

Between coxa i and a short distance from the edge of the shield is a pair of long spines. Running down the centre of the shield are four pairs of short spines.

On the posterior dorsal shield there are seven pairs of spines close to the lateral border, with four long spines on the posterior edge of the shield. There is a pair of small spines near the centre of the anterior margin and a similar pair of small spines about two-thirds of the distance to the posterior end of the shield. On the soft, uncovered integument of the dorsum there is a pair of long spines on each side of the shield.

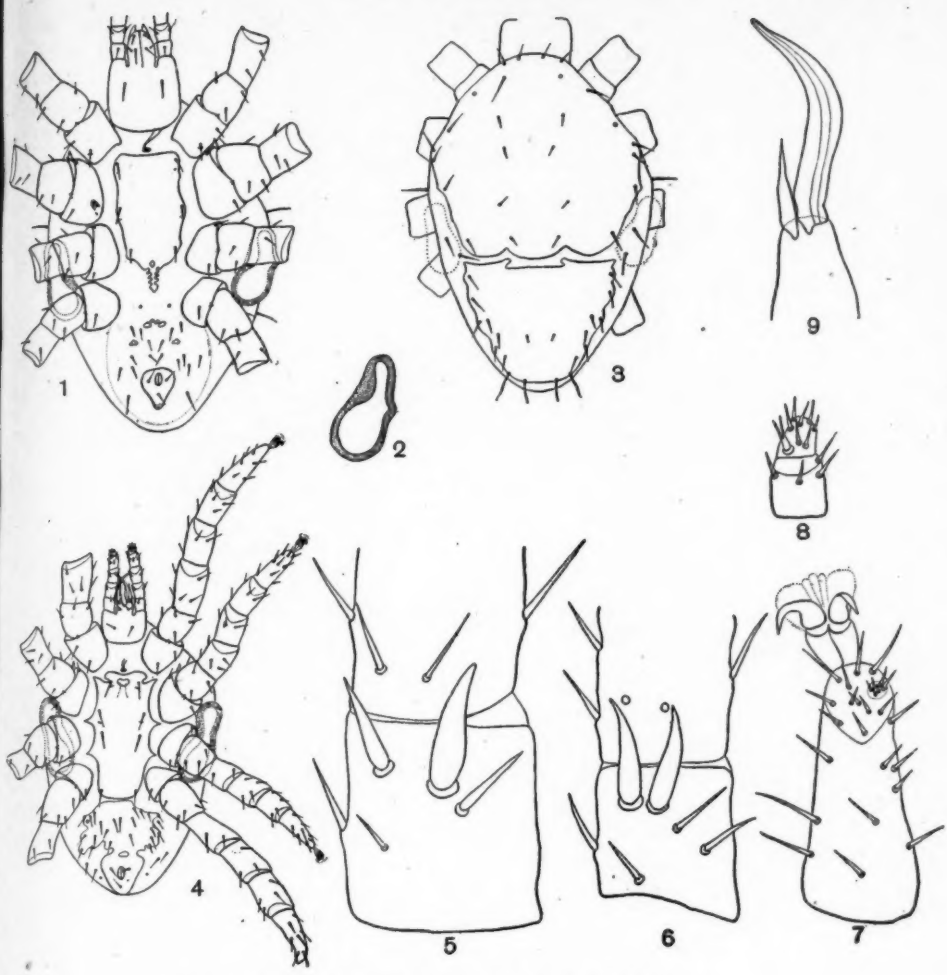


Fig. 1. *Mungosicola ugandae* n.gen., n.sp. Venter of female.
Fig. 2. *Mungosicola ugandae* n.gen., n.sp. Stigmal plate.
Fig. 3. *Mungosicola ugandae* n.gen., n.sp. Dorsum of female.
Fig. 4. *Mungosicola ugandae* n.gen., n.sp. Venter of male.
Fig. 5. *Mungosicola ugandae* n.gen., n.sp. Femur iii, female. Dorsal.
Fig. 6. *Mungosicola ugandae* n.gen., n.sp. Femur iii, male. Dorsal.
Fig. 7. *Mungosicola ugandae* n.gen., n.sp. Tarsus i.
Fig. 8. *Mungosicola ugandae* n.gen., n.sp. 4th and 5th segment of palp.
Fig. 9. *Mungosicola ugandae* n.gen., n.sp. Chelicera of male.

Chaetotaxy of dorsal surface of legs normal except for femur iii (Fig. 5), which bears, in addition to the normal arrangement of spines, a stout tooth-like spine, flanked on one side by a less robust spine, and on the other side a slender spine.

In the Laelaptidae this feature—stout or long spines—is usually present on the dorsal surface of femur i.

On the male venter (Fig. 4) the sterno-genital shield extends from the anterior edge of coxa ii to the posterior edge of coxa iv. The anterior margin of the shield has a central concavity with a deeper concavity on each side flanking the first pair of sternal spines, and antero-lateral corners projecting between coxae i and ii. The lateral borders of the shield bear two concavities with projections between coxae ii and iii, and between iii and iv. From this second lateral projection the shield is extended and rounded beyond the posterior edge of coxa iv.

The shield bears the usual three pairs of sternal spines and in addition the pair of genital spines between coxa iv. The genital pore is placed close to the anterior margin of this shield.

The ventri-anal shield has a very irregular outline, and the spines, numbering about twenty-three (excluding the three anal spines), are placed irregularly on the anterior half of the shield. Posteriorly the shield conforms to the normal anal shield shape—broadly pear-shape. Flanking the anal pore, which lies in the middle of the posterior half of the shield and level with the anterior end of the pore, are the paired anal spines. Midway between the pore and the posterior end of the shield is the unpaired anal spine.

On the soft integument of the body and flanking the anal shield are three spines on each side—in the specimen figured the third spine on each side is missing, but the spine base is easily observed. As in the female there is a large stigmal plate between coxae iii and iv on the lateral edge of the body.

Anterior edge of coxa ii is furnished with a tooth-like spur. Legs robust, i and iv longer than the others. Chaetotaxy of ventral surface of legs normal.

Dorsum of male almost completely covered by dorsal shield. Chaetotaxy of dorsal surface of legs normal except on femur iii (Fig. 6) which differs from that of the female. There are two stout, tooth-like spurs, flanked on one side by two long stout spines. Chaetotaxy of tarsus i similar in both sexes (Fig. 7).

The 4th and 5th segments of palps is shown (Fig. 8). Chelicera of male is seen to have the fixed arm long, stout, sickle-shaped with the movable arm a stout, sharp spur (Fig. 9). Owing to the chelicerae of the two female specimens being turned edgewise it is not possible to see clearly the shape to make a scale drawing, but the chelicerae are, as nearly as possible, as figured (Fig. 10). There are three teeth on the fixed arm and two sharp teeth on the movable arm.

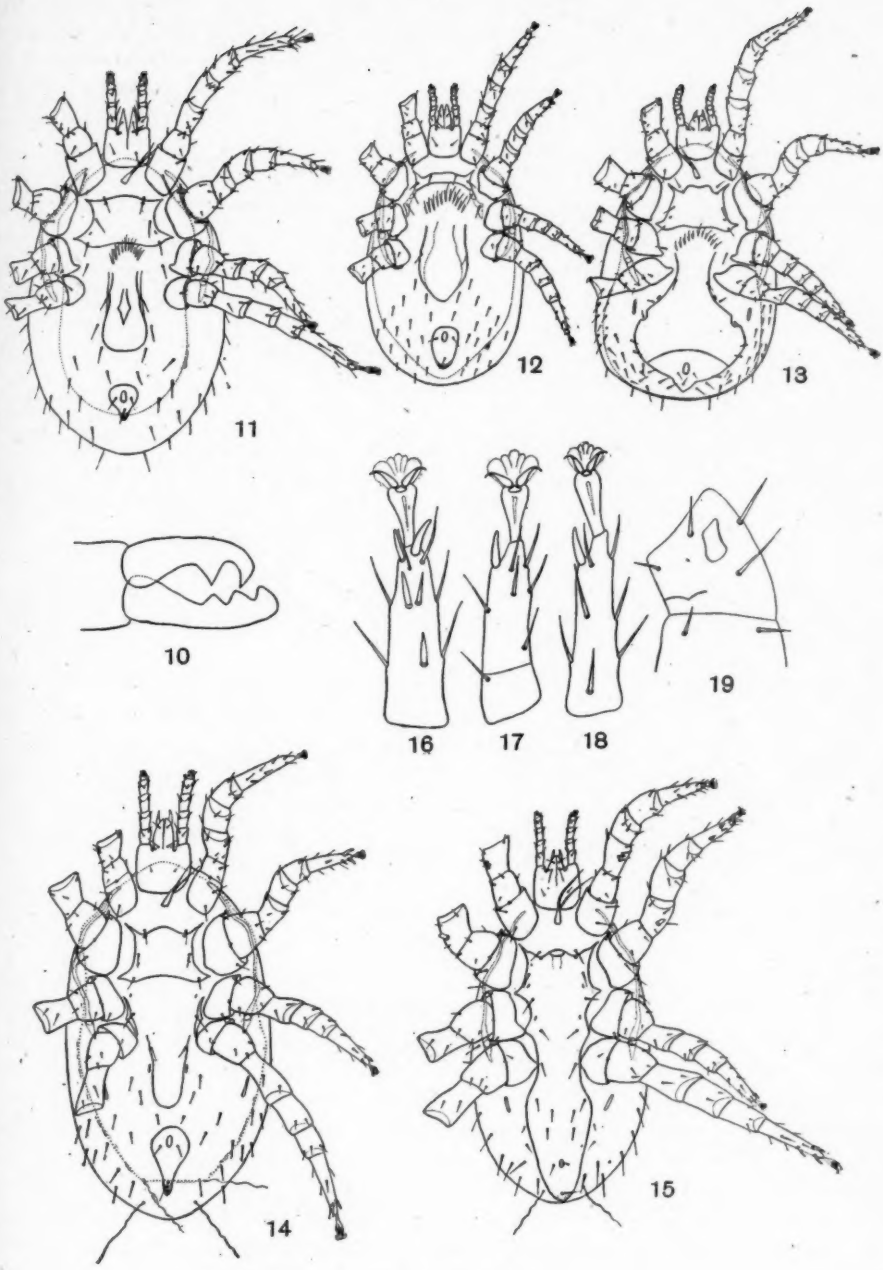
Host. A banded mongoose (*Mungos mungo macrurus* Thomas).

Locality. Kampala, Uganda. 4 March 1940.

Measurements. ♂ 1·0 × 0·7 mm., ♀ 1·2 × 0·8 mm. (without capitulum).

Legends to Figs. 10–19

Fig. 10. *Mungosicola ugandae* n.gen., n.sp. Chelicera of female.
Fig. 11. *Liponyssus sangsteri* n.sp. Venter of female.
Fig. 12. *Liponyssus otomys* n.sp. Venter of female.
Fig. 13. *Ugandolaelaps protoxera* n.gen., n.sp. Venter of female.
Fig. 14. *Haemolaelaps mesopicos* n.sp. Venter of female.
Fig. 15. *Haemolaelaps mesopicos* n.sp. Venter of male.
Fig. 16. *Haemolaelaps mesopicos* n.sp. Tarsus ii of male. Ventral.
Fig. 17. *Haemolaelaps mesopicos* n.sp. Tarsus iii of male. Ventral.
Fig. 18. *Haemolaelaps mesopicos* n.sp. Tarsus iv of male. Ventral.
Fig. 19. *Haemolaelaps mesopicos* n.sp. Femur ii of male. Ventral.



Figs. 10–19.

Genus **Liponyssus** Kolenati, 1858

Liponyssus sangsteri n.sp.

The female venter (Fig. 11) shows the sternal shield to occupy most of the space between coxa ii. The anterior margin is convex medially with antero-lateral projections extending to points between coxae i and ii. Lateral edge of sternal shield concave, postero-lateral corner projected between coxae ii and iii. Posterior border of shield almost straight between the third pair of sternal spines, from whence the shield is curved upward and outward to the postero-lateral corners.

The first pair of sternal spines is placed close to the anterior border of the shield, the second pair midway between the anterior and posterior borders of the shield. The shield also bears two pairs of pores, the first pore lying close to the anterior spine, the second pore between spines ii and iii.

Metasternal shields so weakly chitinized as to be invisible, but metasternal spines present, close to posterior edge of coxa iii.

Genito-ventral shield flask-shaped, extending about one-third of the distance between coxa iv and posterior end of body. Its front edge striated and weakly chitinized, posterior edge almost straight. Genital spines on lateral edge of shield level with coxa iv.

Anal shield pear-shaped, anal pore fairly large with paired anal spines flanking this pore on about its median line. Unpaired anal spine well back towards the posterior end of the shield.

Soft integument of venter furnished with nine pairs of spines which are arranged as figured. A number of spines are situated round the postero-lateral edge of the body, of which the posterior pair are the longest.

Stigmal pore placed between legs iii and iv, peritremal tube extending to middle of coxa i. Legs i and iv longer and more slender than the others, leg ii the stouter. Chaetotaxy of legs normal.

Dorsum of female not completely covered by dorsal scutum which is shaped as shown by the dotted line (Fig. 11).

Material. Several females. Male unknown.

Host. A squirrel (*Protoxerus stangeri centricola* Thomas).

Locality. Bwamba Valley, Toro, Uganda, 1940.

Measurements. ♀ 0·7 × 0·48 mm. (without capitulum).

Liponyssus otomys n.sp.

Sternal shield of female (Fig. 12) placed between coxa ii, anterior border of shield lightly convex medially, with a concavity on each side close to antero-lateral corners which project forward and outward between coxae i and ii. Lateral edge of shield concave, posterior border of shield concave. Postero-lateral corners of shield weakly chitinized which makes the shield appear to terminate level with the middle of coxa ii. This makes it appear that the sternal shield carries only two pairs of spines. The third spine is borne on the weakly chitinized postero-lateral corner.

Metasternal spines borne upon a weakly chitinized shield between coxae ii and iii.

Genito-ventral shield flask-shaped, sharply rounded posteriorly, bearing the genital pair of spines level with coxa iv.

Anal shield broadly oval and placed some distance from the genito-ventral shield.

Anal pore situated in the anterior third of the shield, paired anal spines level with centre of anal pore, unpaired spine in posterior third of shield.

Seventeen pairs of spines are borne on the soft integument of the venter arranged as figured.

Coxa ii bears a strongly chitinized tooth-like spur on its posterior edge. Coxa iii has two tooth-like spurs posteriorly, coxa iv bears a small tooth-like spur which, in some specimens, may be easily overlooked.

Legs somewhat short, chaetotaxy normal with the exception of tarsus ii, which bears two strongly chitinized, short, curved spurs distally. Dorsal shield as shown by the dotted outline.

Host. Water rat (*Otomys* sp.).

Locality. Kisanje, Kigezi, Uganda, 22 June 1940.

Measurements. ♀ 0·57 × 0·38 mm. (without capitulum).

Ugandolaelaps n.gen.

♀. Characters closely similar to *Laelaps*, but having the genito-ventri-anal shield united. Sternal shield broader than long, narrow anteriorly, wider posteriorly and bearing only two pairs of spines and two pairs of pores. Genito-ventral shield flask- or bulb-shaped, narrow neck, broadly convexed laterally and united to the anal shield which is more strongly chitinized. Anal shield with triangular posterior end. One long oval platelet posterior to leg iv.

♂. Unknown.

The genus is raised to include the type species *U. protoxera* n.sp.

Ugandolaelaps protoxera n.sp.

Body broadly oval, broadly rounded posteriorly.

Sternal shield of female (Fig. 13) wider than long, bearing only two pairs of spines and two pairs of pores. Anterior border of shield medially concave, with a deep concavity on each side, lateral corners projecting between coxae i and ii. Lateral edge of shield diverging outward and backward, postero-lateral corners projecting towards space between coxae ii and iii. Posterior border of shield medially convex with a concavity on each side. The first pair of sternal spines is placed in the concavities of anterior border of shield.

Metasternal shields absent or very weakly chitinized, but metasternal spines present, level with coxa iii.

Genito-ventral shield flask-shaped, narrow neck between coxa iv, widening behind. Lateral border of shield broadly convex, then rounded to meet and fuse with the more strongly chitinized anal shield. A small concavity exists laterally at the shoulders of the flask-like shield. Anal shield terminated by a triangular projection. Genital spines borne on lateral edge of genito-ventri-anal shield, level with coxa iv.

Anal shield almost as wide as the genito-ventral shield. Anal pore borne in the centre of anal shield, paired anal spines level with posterior end of pore. Unpaired anal spine situated in the triangular terminal projection.

A small platelet flanks the genito-ventri-anal shield posterior to genital spine, and a long narrow platelet is placed between the shield and the lateral edge of body.

Flanking the shield and posterior to the small platelet there is a small spine, with a

second small spine in the shield concavity. Six pairs of spines are placed close to the ventral shield. On the soft integument of the venter there are eighteen pairs of spines, with a few on the lateral edge of the body with a terminal pair of spines on the posterior end of the body.

Dorsal shield not covering the entire dorsal surface, constricted between coxae iii and iv, leaving the lateral edge of body exposed as shown by the dotted line (Fig. 13).

Legs normal.

Host. A squirrel (*Protoxerus stangeri centricola* Thomas).

Locality. Bwamba Valley, Toro, Uganda, 1940.

Measurements. ♀ 0·6 × 0·43 mm. (without capitulum).

Genus **Haemolaelaps** Berlese, 1910

Haemolaelaps mesopicos n.sp.

The female venter (Fig. 14) shows the anterior border of the sternal shield to be convex medially, flanked by a concavity on each side with the antero-lateral corners projecting forward and outward to the front of coxa ii. Lateral edge of sternal shield concave, posterior border slightly concave, postero-lateral corners projecting towards posterior edge of coxa ii.

The sternal shield bears the customary three pairs of spines and two pairs of pores, though the first pair of sternal spines appear to be placed just anterior to the shield. These spines are borne in the anterior concavity where the chitinization is considerably weaker.

Metasternal shield extending from coxae iii to iv with the metasternal spine level with space between these coxae.

Genito-ventral shield small, narrow, not extending to middle of space from coxa iv to posterior end of body, bearing a pair of genital spines level with coxa iv and flanked by a pair of narrow oval platelets. A long, narrow platelet is present towards the lateral edge of the body posterior to leg iv.

Anal shield elongate, pear-shaped, anal pore in the anterior third of shield, paired anal spines level with the posterior end of pore. Unpaired anal spine close to posterior end of shield.

Three pairs of spines flank the genito-ventral shield, and ten similar pairs of spines are placed on the soft integument of the venter. Body terminated posteriorly by a pair of long, sinuous spines.

Chaetotaxy of legs normal except for tarsus ii which bears a stout, tooth-like spine in the centre and two stout spines distally on the ventral surface. Tarsus iii and iv bearing a stout spur distally on the anterior edge.

Dorsal shield not covering dorsum, leaving a narrow uncovered border. Posterior end of dorsal shield straight between the two long, sinuous spines which terminate this shield. Spines on dorsum few, those in middle of shield short, small, those on lateral border of shield longer. Outline of shield shown by dotted line (Fig. 14).

The male venter (Fig. 15) shows the anterior border of the shield to be similarly shaped to that of the female, with a medial convexity flanked by a concavity on each side, antero-lateral corners diverging outward and forward.

Shield concaved laterally to a projection between coxae ii and iii, again concaved

with a second lateral projection level with posterior edge of coxa iii, a third concavity round coxa iv, whence the shield converges to a broadly rounded end posteriorly.

Genital pore situated on the convexed anterior border of shield. The customary three pairs of sternal spines and two pairs of pores are followed by the metapodal pair of spines. Lying between coxa iii and anterior to the metapodal spines there is a third pair of pores. Genital pair of spines level with coxa iv, followed posteriorly by three pairs of spines. Anal pore placed some distance before the posterior end of shield, flanked by the paired anal spines. The unpaired anal spine is close to the posterior end of shield.

Flanking the shield midway between it and the lateral edge of body and posterior to leg iv is an elongated, elliptical platelet. Some ten pairs of spines are situated on the soft integument of the body, posterior to leg iv, two pairs of which are situated close to the lateral edge of the shield. A pair of very long, sinuous spines is placed on a level with the unpaired anal spine.

Chaetotaxy of legs normal except for femurs i and ii, and tarsi ii, iii and iv. On the venter of femur i there is a group of three stout spines. On femur ii there is a stout, truncated spur. Tarsus ii possesses two or three stout spines, and is terminated distally by two stout, curved spurs.

On tarsi iii and iv there is a stout spur on the anterior distal edge (Figs. 16–19).

Dorsal shield covering the dorsum, chaetotaxy as in the female.

Host. A woodpecker (*Mesopicos ruwenzorii* Sharpe).

Locality. Kabale, Kigezi, Uganda, 15 August 1940.

Measurements. ♀ 0·85 × 0·44 mm., ♂ 0·73 × 0·42 mm. (without capitulum).

Material. 4 ♂, 3 ♀.

TECHNIQUE

Having been requested by a number of acarologists for a description of the methods adopted in the preparation of my microscopic slides, I feel that it may be of interest to describe it here.

In the early days of my work I used sodium hydroxide to clear the specimens, but on the recommendation of Dr Ivar Tragardh, the eminent Swedish acarologist, I started to use lactic acid.

The specimens are placed in a glass staining cell with a small quantity of lactic acid, enough to cover the specimens. The cell is then warmed for a period of 5–10 min., care being taken to prevent the heat being excessive. The cell is then examined under a low-power 2 in. objective. Each specimen is pressed down in the acid to insure complete immersion, as there is a tendency for the specimens to float on the surface.

A further warming is given and the process continued until the specimens are transparent. Those specimens that are transparent are transferred to a second staining cell containing equal parts of water and gum chloral (De Faure's medium). The specimens are left in this second cell for 2 or 3 hr. to thoroughly dissolve the lactic acid, which, if not cleared from the specimens will crystallize when the slide is dried, spoiling the appearance of the mount.

The specimens are now ready to be mounted direct on the microscope slide in De Faure.

Weakly chitinized specimens, particularly the endoparasitic genera—*Pneumonyssus*, *Entonyssus*, *Cytolichus* and the Sarcoptidae—need careful treatment owing to the thinner chitin. The Laelaptidae, being more strongly chitinized, require longer treatment to make

them transparent, and occasionally one may find it necessary to press out the contents of the body with a blunt needle.

A number of stains have been tried, but my preference is acid fuchsin. This can be applied to the first staining cell, making the clearing and staining one process, or used as a separate process after clearing the specimens in the lactic acid.

The quantities of lactic acid, stain and De Faure cannot be stated, one has to experiment. Too much stain clogs up the specimens and care should be taken in using it. A second stain is not usually necessary with the mites.

The excess De Faure can be wiped off after the cover-glass has been applied to the mount, and the slide placed on a tray in the drying oven.

Ringing cements of various brands have been tried, but it has been found that 'Euparal' is the best for use with De Faure's mountant. If a second (coloured) cement is desired, brown or white ringing cement can be applied on the top of the 'Euparal' ring, which, of course, must have been dried before applying the second ring.

For suggestions in the staining and ringing processes I have to acknowledge my thanks to Mr Harry Britten, F.R.E.S., Manchester.

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THE MECHANISM OF INSEMINATION IN THE ARGASID TICK, *ORNITHODORUS MOUBATA* MURRAY

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(With 1 Figure in the Text)

Introduction

During the raising of a fairly large stock of the tick *Ornithodorus moubata*, for the purposes of insecticidal tests, egg viability suddenly decreased alarmingly. It therefore became necessary to study factors which might influence development of the eggs, the first and most important of which is the presence or absence of sperms in the oviduct of the female. This paper deals with the process of insemination and the fate of the sperms.

The process of insemination

Nuttall & Merriman (1911) fully describe the behaviour of *Ornithodorus moubata* during mating, and all that need be repeated here is that the male crawls underneath the female and clings to her so that their ventral surfaces are together. After mutual stimulation the male produces a spermatophore from his genital opening, grasps it with his chelicerae, and transfers it to the vulva of the female. At the same time he secretes a quantity of coxal fluid.

The anatomy of other ticks has been studied by Nordenskiöld (1906*a*, *b*) and Samson (1909*a*, *b*), both working on species of *Ixodes*; Allen (1905) and Williams (1905), both working on *Boöphilus*; Robinson & Davidson (1913), working on *Argas persicus*. Of these authors only Samson (1909*a*) mentions mating in any detail, but she concludes that the entry of the male proboscis into the female vulva has nothing to do with insemination. The description of her single observation on mating in *Ixodes ricinus* is so like that of Nuttall & Merriman for *Ornithodorus moubata*, that there is little doubt of the process being essentially similar in the two ticks.

In the male *Ornithodorus* the spermatids may travel down the vasa deferentia in a continuous stream or aggregated in rounded pellets each containing a few hundred male elements. It is presumably the male accessory glands (Robinson & Davidson, 1913) which secrete the spermatophore-case.

A complete spermatophore, after use, consists of a bulb about 1 mm. in diameter with a long slender neck which bears two capsules at its end each many times larger than the bulb (Fig. 1 B). Contrary to the statement of Nuttall & Merriman the spermatophore is not chitinous as it completely dissolves in strong KOH solution at 150° C. It becomes red in Millon's reagent, and therefore is probably largely protein.

The discharge mechanism is complicated. Just before insemination, the bulb only is pushed out of the male aperture and is filled with a colourless liquid. Very soon afterwards the capsules and neck containing the spermatids, hitherto still in the ductus ejaculatorius, are forcibly squeezed into the bulb which consequently stretches considerably, and now looks white and opaque (Fig. 1 A). The lips of the male aperture grip the small length of neck which remains external to the bulb (Fig. 1 A, neck). The male next takes the spermatophore, holding it by the neck with his chelicerae, and inserts

his proboscis into the vulva of the female. The bulb remains outside and, on relaxation of the male's grasp, its tension causes it to contract and push out the capsules into the genital tract of the female. After the bulb contracts it dries to an almost flat disk, and the spermatids are carried up the neck into the capsules which now fill the uterus, each pressing into the lower part of the corresponding oviduct. If a spermatophore be taken from the male, it will usually evaginate spontaneously; it will do this in air or immersed in Ringer's fluid, so that mechanical tension seems to be the motive force, and not

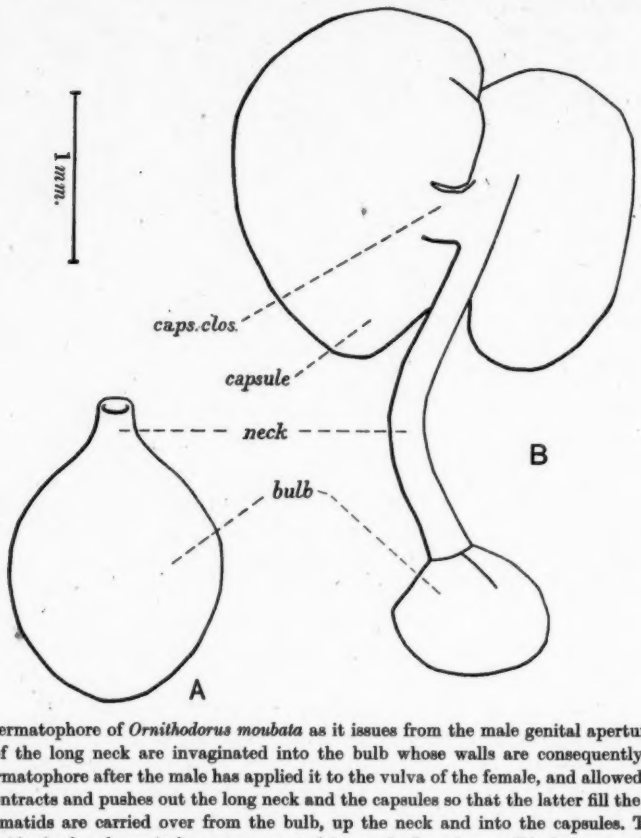

Fig. 1. A. A spermatophore of *Ornithodorus moubata* as it issues from the male genital aperture. The capsules and most of the long neck are invaginated into the bulb whose walls are consequently under tension. B. The spermatophore after the male has applied it to the vulva of the female, and allowed it to evaginate. The bulb contracts and pushes out the long neck and the capsules so that the latter fill the uterus. Almost all the spermatids are carried over from the bulb, up the neck and into the capsules. The bulb, which remains outside the female genital aperture, soon dries, and often drops off before oviposition. *Caps.clos.* capsule closure.

shrinkage due to drying, or osmotic pressure. Successful insemination can take place even if the uterus is crowded with eggs, but on occasions the male allows the capsules to evaginate uselessly outside the body of the female owing to faulty manipulation. Nuttall & Merriman saw only the bulb and part of the neck of the spermatophore, and wrongly assumed that the seminal fluid was discharged free into the vagina when the ticks pressed the bulb flat after the male had broken the tip of its neck. These authors suggest that the male coxal fluid helps to keep the bulb soft so that it can easily be squeezed empty. When present it may indeed help in the efficient contraction of the

bulb, but the male does not invariably pour out this fluid during copulation. The coxal pores of some males were blocked variously with plasticine, hot wax and rubber solution. Six out of eight attempted and successfully completed copulation with this enforced absence of coxal fluid. Certain stimuli such as local application of heat or rough handling cause all stages of the tick to produce a quantity of the fluid, and its appearance during copulation seems to be incidental to sexual excitement. Clearly it is not a physiological necessity for successful insemination.

After copulation the bulb soon becomes dry and brittle and usually drops off with a length of neck attached. The remains of the neck on each of the two capsules contracts in a twisted fashion and makes an effective seal (Fig. 1B, *caps.clos.*). This can be tested by putting a capsule in distilled water: it swells up by osmosis which could only occur if the seal were good.

The spermatids mature in the capsules inside the uterus in about 5 days at 30° C., and escape as sperms by rupture of the capsule wall. This may happen when the female feeds or when eggs enter the uterus. As many as ten empty capsules have been seen in one female. The sperms which are relatively large and slow moving (Christophers, 1906) are somewhat less than 0·5 mm. in length, i.e. about half the length of the body of a newly emerged first stage nymph. Samson (1909*b*) erroneously states that the mature sperms have only a short existence prior to fertilization, passing most of their existence in the immature form. The sperms can be found free in the uterus, but the majority enter the oviducts and travel as far as the junction of the lower wide section with the upper narrow one, adhering especially just below this junction. After oviposition is completed, sperms are very difficult to find in the female genital tract, and it would seem that for 100% fertility in second and subsequent batches of eggs, corresponding inseminations are necessary. Jobling (1925) mentions that under experimental conditions the viability of later batches of eggs is lower than that of the first two or three batches. He correlates this with the general decline in the activity of the female tick after two or three feeds, and states that he always found a few sperms present when he dissected females which had mated once and oviposited more than once. Future work will show whether one insemination is enough for the full fertility of more than one batch of eggs.

An interesting comparison may be made between the process in *Ornithodorus moubata* and those observed by Michael (1892) in *Haemogamasus hirsutus* and other genera in the Mesostigmata *Canestrini* division of the Parasitiformes *Reuter*. Michael records that 'a hyaline bubble comes out of the male aperture during mating and rapidly swells until it is as big as the body of the male. A small neck remains in the male aperture. Later a thickish fluid with spermatocysts enters the hitherto clear spermatic capsule. The male then grasps the neck of the latter and applies it to the female with his mandibles. The elasticity of the capsule drives the slight stopping out of the neck and the contents discharge. If the creatures are parted before the capsule has discharged, it will probably do so of its own accord.' In common with the spermatophore of *Ornithodorus moubata*, that of *Haemogamasus hirsutus* first appears at the male aperture as a small clear bulb which increases in size to accommodate the seminal fluid. The males in both cases use their proboscis to apply the spermatophore to the female opening, and it discharges by virtue of its mechanical tension. Michael does not mention any structures corresponding to the capsules in *Ornithodorus*, but the similarity of the processes in all other respects suggests that they may be present also in *Haemogamasus*.

SUMMARY

The spermatophore in *Ornithodorus moubata* consists of a bulb with a neck at the end of which are two large capsules. When the spermatophore issues from the male opening the capsules are invaginated into the bulb to form a compact but tense object which the male applies to the female aperture with his proboscis. The male releases his grip on the neck, and the tension in the walls of the bulb causes the capsules to evaginate and thus enter the uterus of the female.

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SOME HAEMATOZOA OBSERVED IN VERTEBRATES IN EASTERN CANADA

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(With Plates II–IV)

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Introduction

This paper is the fourth of the series embodying results of some of our observations on parasitic Protozoa and Helminthes in eastern Canada. It includes work on blood-inhabiting Flagellata and Sporozoa, together with a *Microfilaria*. Much time has been spent in consulting the voluminous literature on Haematozoa, much of which, necessarily, is of an incomplete nature. The classification, particularly of the Sporozoa, is still in a state of flux, due to further discoveries in connexion with the life cycles of members of certain genera. Owing to war exigencies, both text and illustrations have been condensed and discussion of systematics reduced as far as possible to that of nearly related forms.

Material and methods

The hosts of the Haematozoa described are fishes and Amphibia. The fresh-water fish were obtained from the St Lawrence River and streams and lakes in Quebec Province. The salt-water fish were caught at St Andrews, New Brunswick, or at Halifax, Nova

Scotia, the two places being about 250 miles apart by sea, while a few fish were bought in Montreal. The Amphibia were collected in Quebec Province.

Visits to some sites on the first occasion yielded many hosts. On subsequent visits none were found, owing to the activities of collectors of frogs for food, who removed the legs and left the rest of the cadavers to decay, or to depredations of herons and kingfishers, which almost cleared some fresh waters of fish. Some tadpoles were raised from spawn.

Hanging-drop preparations of fresh whole or citrated blood were always examined, also stained blood smears and teased or smeared organ preparations. The amount of infected material seldom allowed of sections. Dry fixation and wet fixation with osmic acid vapour, formalin, Schaudinn and Carnoy fluids have been employed. Heidenhain iron haematoxylin, Rosenbusch and Delafield haematoxylin, Giemsa and Leishman stains have been used, with neutral red, azur II in saline and methyl green as intra vitam stains. Green light and dark-ground illumination have been useful. All drawings were made with a camera lucida.

The new specific names proposed are arbitrary combinations of letters or barbarous names, and therefore, in the spirit of Articles 8 (*k*) and 14 of the International Rules of Zoological Nomenclature, are not subject to change.

The Haematozoa observed

The blood-inhabiting Mastigophora comprise five trypanosomes new to science and some known ones, and a new spirochaete is appended. The Sporozoa include hitherto undescribed species of *Lankesterella*, *Haemogregarina*, *Plasmodium*, *Haemoproteus*, *Leucocytozoon* and *Dactylosoma*. The only haematozoic helminth is a new *Microfilaria*.

MASTIGOPHORA

Two piscine and five amphibian *Trypanosoma* are described.

Trypanosoma percae n.var. **canadensis** (Pl. II, figs. 1, 2)

Trypanosoma percae n.var. *canadensis* has been found in small numbers in the blood and organ smears of one yellow perch, *Perca flavescens*, out of a collection of fifty-three taken on the same date by a fishing party. Single blood smears of over 200 yellow perch have been examined from many parts of Quebec with negative results, though extremely light infections may not have been detected.

The trypanosome in life is relatively transparent. It is actively motile, usually moving with the anterior end forwardly directed, though it can move with the posterior or aflagellar end forwards. The free flagellum is short but active. Using green light or a dark-ground condenser, both body and membrane myonemes could be more easily seen. In fresh preparations the trypanosomes appeared to be uniform in size, and in stained preparations showed only a small dimensional range.

The body of the trypanosome (Figs. 1, 2) is sinuous, with both ends tapering, the posterior being more acute than the anterior end. Passing down both surfaces of the body are 8–10 spirally arranged myonemes. The endoplasm contains a few larger, deeper staining granules. The nucleus is rather large, ovoid (Fig. 1) to oval (Fig. 2) in outline. In stained specimens it is usually homogeneous (Fig. 1), but occasionally an indication of a karyosome (Fig. 2) has been noted. The kinetoplast (or parabasal body) is small

and bar-like, situated relatively near the posterior end of the body. Near the kinetoplast is a distinct dot-like, rounded basal granule, near which the flagellum arises. This borders the folded undulating membrane and has a short free portion. The undulating membrane also has myonemes, but these are much less prominent than those of the body. Dividing forms have not been observed.

The dimensions of the trypanosome are: body length, 28·9–32·5 μ; body breadth, 3·4–4·2 μ; free flagellum, 5·3–6·9 μ; nucleus, 3·1–4·1 by 2·1–2·3 μ.

Trypanosoma percae, from the European perch, *Perca fluviatilis*, was first noted by Brumpt (1906), who gave a brief note on it, with dimensions. He stated it was 57 μ in total length, of which 16 μ was free flagellum, body breadth 3 μ, kinetoplast 1·5 μ from the posterior end and nucleus situated equidistant from the root of the flagellum and the posterior end. Minchin (1909) found a trypanosome in *P. fluviatilis* in Norfolk, England, which he identified as *Trypanosoma percae* 'solely on the ground of occurrence', as Brumpt's description 'fails to note any of the peculiarities of the trypanosome of the perch'. Minchin himself gave a very detailed account of the behaviour and cytology of the perch trypanosome. When two of us first saw the trypanosome of the yellow perch in Canada, it at once recalled Minchin's beautiful preparations that we had seen in England. Comparison of the two organisms, however, showed some differences. The Canadian trypanosome is monomorphic, its body is shorter and broader and its free flagellum is always relatively short, while it is short in the stout English trypanosomes and long in the small forms. While there are these constant differences, there are general resemblances with *T. percae* as described by Minchin. The trypanosome of *Perca flavescens* is nearly related to *Trypanosoma percae* of *Perca fluviatilis*, but is separable by the differences noted. As far as we can ascertain, the host is new and the trypanosome is recorded for the first time from Canada. It is proposed, therefore, to name it *Trypanosoma percae* n.var. *canadensis*, with characters as herein described.

Trypanosoma myoxocephali n.sp. (Pl. II, fig. 3)

Trypanosoma myoxocephali n.sp., an organism apparently new to science, has been observed in the blood of one out of five longhorn sculpins, *Myoxocephalus octodecimspinosus*, caught at Halifax, Nova Scotia. Five from St Andrews, New Brunswick, were unparasitized. The infection was slight. No ectoparasites were present.

In life, the trypanosome is active, often flexing its body in a crook-like manner. The membrane is relatively straight, bordering the body, and two myonemes can be seen by dark-ground illumination. The trypanosomes are monomorphic and vary little in size or appearance. Smears of the heart blood of the fish contained most trypanosomes, organ smears much smaller numbers.

The trypanosomes stain well (Fig. 3). The body is usually curved into a crook, with the posterior end more acute than the anterior flagellar end. The cytoplasm stains rather deeply with Giemsa stain and three myoneme fibrillae, best seen in the part of the body anterior to the nucleus, are present. The nucleus, situated in the posterior half of the body, is bean-shaped to oval, with evenly distributed chromatin granules. A few chromatoid granules are present between the nucleus and the rather large kinetoplast. The undulating membrane bordering the body has a thick flagellum that originates near a round basal granule and terminates anteriorly as a short, free flagellum.

The dimensions of the trypanosome are: body length, 41·5–49 μ; body breadth, 3 μ; nucleus 6·7–7·7 by 2·2–3 μ; free flagellum, about 3 μ.

Two dividing forms only have been seen in a kidney smear. In one, two nuclei, two kinetoplasts and a partly divided undulating membrane were present; in the other cytoplasmic cleavage had also begun.

Many trypanosomes have been recorded from various marine fishes. As far as we can ascertain, this is the first record from any species of *Myoxocephalus*. Having compared descriptions and illustrations as far as present conditions permit, we can find no form exactly corresponding to this trypanosome, which differs from most marine fish trypanosomes in its smaller size, narrower undulating membrane and short free flagellum. It is, therefore, designated *Trypanosoma myoxocephali* n.sp., with characters as herein outlined.

Trypanosoma lavalia n.sp. (Pl. II, fig. 4)

A new trypanosome has been observed in small numbers in the blood of one *Bufo americanus* captured at Laval des Rapides, P. Que.

The trypanosome (Fig. 4) is delicate in appearance, active in movement on the spot, swirling round with slight progression. It always assumes a characteristic reflexed form. The undulating membrane is broad and is markedly vibratile. The free flagellum, though very short, can be seen in life.

The curved body of the trypanosome stains fairly deeply, but chromatoid granules are absent from the cytoplasm. The anterior flagellar end is markedly acuminate, the posterior end much broader and acute. The ovoid, granular nucleus is situated nearer the posterior end. The kinetoplast is oval, situated very near the posterior end of the body, with its long axis parallel to that of the body. A basal granule is near it. The undulating membrane is as wide or wider than the body and two myonemes have been observed.

The dimensions of the trypanosome are: body length excluding free flagellum, 31·1–35·5 μ; body breadth, 3·9–4·4 μ; nucleus, 3·7–4·1 by 2·6–3 μ; free flagellum, 1·5–2·6 μ.

Multiplication by binary longitudinal fission has been seen occasionally in stained preparations.

This trypanosome of *B. americanus* recalls somewhat one we have observed in the blood of *B. regularis* in the Transvaal, but the Canadian parasite is smaller, has a more prominent membrane with only two myonemes, an oval prominent kinetoplast parallel to the long axis of the body, and a very much shorter free flagellum. The same features distinguish it from the unnamed and undescribed trypanosome, depicted by Balfour in 1908, from *B. regularis* in Khartoum, which also differs from the one we have examined from the same host in the Transvaal. *Trypanosoma bocagei* França, 1910, from *Bufo regularis* in Portuguese Guinea is much smaller than our Canadian trypanosome, being only 16·5–18 μ long, while the kinetoplast is often superimposed on the nucleus or cannot be distinguished from it, as França has pointed out. Mathis & Léger (1911) have described *Trypanosoma bocagei* from *Bufo melanostictus* in Tonkin, but this had a free flagellum about 35 μ long. These features separate the trypanosome of *B. americanus* from *Trypanosoma bocagei*. The foregoing appear to be the nearest affinities to the trypanosome here described, but it is distinct from them. It is, therefore, named *Trypanosoma lavalia* n.sp., with characters as herein summarized.

Trypanosoma gaumontis n.sp. (Pl. II, figs. 5, 6)

A small trypanosome has been found as a sparse infection in the blood of one out of five *Bufo americanus* collected from the verges of Lac Gaumont, P. Que.

In life the trypanosome is slow-moving, though its undulating membrane vibrates vigorously. The body usually has a crook-like curve. Neither a free flagellum nor body myonemes can be detected, even with dark-ground illumination, but a single myoneme, parallel to and very near the bordering flagellum, has been observed, and at times the illusion of a double border is created. Cytoplasmic vacuoles have not been observed, either in living or stained trypanosomes.

In stained preparations, the anterior flagellar end of the trypanosome is attenuate, the posterior end more acute, and the body often broadens in the neighbourhood of the kinetoplast (Figs. 5, 6). The oval, granular nucleus is situated near the posterior end of the body. The undulating membrane has deep folds and one myoneme very near and parallel to the bordering flagellum. There is no free flagellum.

The measurements of all the trypanosomes observed fall into two groups, one 15–15·8 by 1·3–1·85 μ, the other 19·7–20·7 by 1·5–1·85 μ. The nucleus measures 1·5–2·2 by 1·1–1·5 μ.

Similar forms have been observed in organ smears. Dividing forms were rare.

Ectoparasites were not present either on the host or on four uninfected toads caught at the same time. No sanguivorous aquatic insects or leeches were obtained and dissection of some of the voracious blackfly, *Simulium venustum*, caught nearby, revealed no stages of trypanosomes. The mode of transmission remains unknown.

This trypanosome is of the small type. It is separated from *Trypanosoma parvum* Kudo, 1922, parasitic in *Rana clamitans*, as it is smaller, its cytoplasm is not vacuolated, its nucleus is nearer the posterior and not the anterior end of the body, and it has no free flagellum. Also, in contrast with *Trypanosoma parvum*, it is slow in movement. It occurs in a new host, in a new locality. As far as we can ascertain, no such trypanosome has been recorded previously from Amphibia. It appears to be a new species and is, therefore, designated *Trypanosoma gaumontis* n.sp., with characters as herein set forth.

Trypanosoma montrealis n.sp. (Pl. II, figs. 7–10)

A trypanosome, which appears to be a new species, occurred in the blood of three *Bufo americanus* out of about two hundred collected over a period of five years on or about Mount Royal, Montreal. The infections were not heavy.

The flagellate (Figs. 7–10) is a long, sinuous organism, with a narrow but distinct undulating membrane. Both ends of the body taper (Figs. 7, 8), though in some the posterior end is somewhat more sharply pointed than the anterior flagellar end (Figs. 9, 10). The endoplasm is finely granular and free from larger chromatoid granules. The nucleus is roughly oval in outline, with evenly distributed chromatin, and is often situated near the middle of the body, sometimes rather nearer one end. The kinetoplast is small but distinct, and between it and the root of the flagellum a basal granule is present (Figs. 8, 9). The undulating membrane is rather narrow and is often contracted close to the body. The free flagellum is always very short.

The dimensions of this trypanosome are: body length, 45–68 μ; body breadth, 1·8–6 μ; nucleus 3–6 by 1·8–3·7 μ; free flagellum, 3–5·5 μ. Most of the trypanosomes were 45–55 μ in body length. A few dividing forms have been observed.

Regarding the affinities of this trypanosome, its dimensions, position of the kinetoplast, short free flagellum and straighter membrane separate it from *Trypanosoma inopinatum* Sergent & Sergent, 1904. It differs from *T. nelspruitense* Laveran, 1904, which has marked chromatoid cytoplasmic granulations and a very long free flagellum. *T. bocagei* França, 1910, redescribed by Mathis & Léger from *Bufo melanostictus* in Tonkin (1911), has large and small forms, the large ones being somewhat of the same dimensions as ours. But *Trypanosoma bocagei* is markedly polymorphic, its undulating membrane is wide, its posterior end more acute, its free flagellum longer and its nucleus circular. *T. chattoni* Mathis & Léger, also from Tonkin toads, is smaller and has a much longer free flagellum than the Canadian trypanosome. The trypanosome of *Bufo americanus* from Mount Royal seems a new species. It is, therefore, named *Trypanosoma montrealis* n.sp., with characters as outlined here.

Trypanosoma rotatorium (Mayer) (Pl. II, figs. 11–18)

Trypanosoma rotatorium has been found by us in the blood and organ smears of *Rana catesbiana* from Montreal, Old Mill (Back River), Lantier, Foster, St Eustache, Bordeaux and Lake Manitou; *R. clamitans* from Montreal, Lantier and Lac Nominogue; and *R. pipiens* from Montreal, Lac Gaumont and Labelle City. Flagellate and non-flagellate forms have been observed in life, together with transitional forms between the two, and the formation in vivo of the non-flagellate stage or latent body. Blood and organ smears have been examined intensively.

There is little difference in general morphology of *Trypanosoma rotatorium* from Canadian Amphibia and those from Europe and South Africa. The trypanosome from Canadian frogs (Figs. 11–13) is markedly polymorphic, small and large, broad and narrow forms occurring in the same host. The anterior end is narrow and pointed, the posterior end also pointed but more abrupt, and, in some, may even be more or less rounded. The body has well-marked myonemes and the cytoplasm is densely granular. The undulating membrane is conspicuous, with many folds, has myonemes, and is bordered by the strong flagellum. The free part of the flagellum is very short (Figs. 12, 13); in fact, there may be hardly any free flagellum or none (Fig. 11). The nucleus is relatively small, oval or rounded in outline and shows cyclical variation in structure. It may be karyosomatic (Fig. 11) or with many fine chromatin granules (Figs. 12, 13) and is often difficult to stain well. It is situated usually to one side of the body, near the undulating membrane. The kinetoplast or parabasal body is often small and surrounded by a clearer area (Figs. 11, 13) and may be dot-like (Fig. 11) or rod-like (Fig. 13). The flagellum originates near the kinetoplast. Occasionally a minute basal granule between the origin of the flagellum and the kinetoplast has been seen. Organ smears usually contained more trypanosomes than blood smears.

T. rotatorium in all stages of division has been observed in the living condition and in stained preparations. More dividing forms were counted in smears of the kidney than in any other organ, much smaller numbers occurred in the spleen, and occasionally some were found in heart blood and liver preparations. Trypanosomes in various stages of binary fission were usual (Fig. 14); sometimes cases of incomplete separation followed by a second or third division were observed (Fig. 15), such eventually resulting in four and eight trypanosomes. Subequal binary fission was fairly common.

Stages of rounding or formation of latent, resistant or non-flagellate phases have been observed in vivo in preparations of the spleen and heart blood, and studied also in smears of these organs and in those of liver and lung. The process may be summarized as comprising a general contraction of the body of the trypanosome into a more or less rounded mass, to which the undulating membrane at first is very closely adherent (Fig. 16) and into which it may become largely or completely absorbed (Figs. 17, 18); disintegration of the myonemes into shorter curved rows or areas of deep-staining granules; migration of the kinetoplast nearer to the nucleus (Figs. 17, 18), on which it may or may not become superimposed; and, consequent on changes in membrane and myonemes, together with cytoplasmic contraction, a densely granular appearance of the resistant body (Fig. 18). Nöller (1913, 1913*a*) has described rounding and rounded forms of *T. rotatorium* from European frogs, and two of us have seen the same in *T. rotatorium* from *Xenopus laevis* and *Rana fuscigula* in South Africa.

In Canada, the narrower forms of *Trypanosoma rotatorium* have been observed in tadpoles of *Rana catesbiana* and *R. clamitans*. Examination of one part of a batch of year-old tadpoles of *R. catesbiana* (the larval stage of which covers two years), showed only the ordinary flagellate forms of *Trypanosoma rotatorium* in heart and organ smears. Another part of this batch of tadpoles was kept in an aquarium and fed on calves' liver. At twenty-two months, several were killed. Extremely few motile trypanosomes were present in the preparations, but the spleens contained some rounded forms. Only three metamorphosed into the adult frog and, of these, two showed flagellate forms and one rounded forms and very few flagellate forms. It would seem that the rounded forms are truly latent and capable of resisting the strain of metamorphosis. The break-up of the team of observers due to the lamented death of the senior author and the departure of both remaining partners for other spheres of work has prevented repetition and elaboration of these suggestive observations.

It may be mentioned that the dimensions of *Trypanosoma rotatorium*, as observed in Canadian frogs, vary much, due to its polymorphism. Thus, the body length is 47·4–72·6 μ; body breadth, 3–26·7 μ; nucleus 3–6·6 by 2·2–5·9 μ; free flagellum, when present, 1·5–8·5 μ. Trypanosomes from *Rana catesbiana* and *R. clamitans* were rather longer than those from *R. pipiens*, and those from tadpoles usually more slender than those from adult frogs.

Transmission experiments with leeches and Culicines gave negative results, but not many could be attempted.

Trypanosoma inopinatum Ed. & Et. Sergent, 1904

We record the occurrence of a trypanosome morphologically identical with *Trypanosoma inopinatum* Ed. & Et. Sergent, from the blood of small numbers of *Rana catesbiana* and *R. pipiens* from the environs of Montreal. In no case was the infection heavy. As in Europe, *Trypanosoma inopinatum* appeared to be non-pathogenic to its hosts, whereas in Algiers it is lethal.

A form corresponding in dimensions and morphology with *T. inopinatum*, except that its free flagellum is only 1·5–2·5 μ in length, has been found by us in the blood of *Rana catesbiana* from St Eustache.

Spirochaeta manitoui n.sp. (Pl. II, figs. 19, 20)

A spirochaete sens lat. has been observed in small numbers in the heart blood of one *Rana catesbiana* collected at Lake Manitou, P. Que. Being well aware of the occurrence of numerous organisms in water, great care was taken, as usual, to avoid any contamination. The organisms were not very numerous but moved actively in fresh preparations. With dark-ground illumination, refractile bars or granules of the distributed nucleus could be detected in some specimens. The number and depth of the coils varied with the activity of the organism.

One end of the spirochaete (Figs. 19, 20) is rounded, the other tapers. Using magnifications of over 3000, a trace of a membrane seemed to be present. At magnification of 1800, stained chromatoid bars, some 12–15 in number, could be seen. The spirochaetes measured 12·5–16·5 μ in length and 0·2–0·3 μ in breadth. Actual division was not seen in life, but a few forms showing indications of transverse binary fission have been observed.

Examination of organ smears of the infected host revealed very few spirochaetes. Small *Spirillum* were present in the rectal contents but never showed more than two coils and were quite unlike the organism from the blood. The centrifuge deposit from a sample of the water from Lake Manitou contained many flagellates, ciliates and bacteria, but nothing corresponding to the blood spirochaete. Some young *Lymnaea stagnalis* were collected on the same occasion but no spirochaete was found on dissecting them.

The spirochaete is much longer than the gut spirochaete that has been described from Amphibia, especially from toads, by a number of workers. It appears to have no ill effect on its host. For the present, it is designated *Spirochaeta manitoui* n.sp., with characters as described, for purposes of reference.

SPOROZOA

The classification of the blood-inhabiting Sporozoa is difficult, but, at best, classification only marks the state of knowledge for the time being. Many organisms, formerly placed in the genus *Haemogregarina*, have now been removed therefrom, either because research has shown differences in their life cycles or because of analogies with various phases of other ascertained life cycles. The present use of the name *Haemogregarina*, then, is in the broad sense, and we have sympathy with the comment of Reichenow in his revision of Doflein's *Lehrbuch der Protozoenkunde*, p. 925 (1927–9) that the name *Haemogregarina* is largely a repository for insufficiently known forms.

The genera *Plasmodium* and *Haemoproteus*, as defined by Wenyon (1926), were separated largely by the schizogony of *Plasmodium* occurring in the host erythrocytes and that of *Haemoproteus* in the endothelial cells of organs. During the last seven years, much work on the species of *Plasmodium* infecting birds has shown the occurrence of exo-erythrocytic schizogony of at least five species in the reticulo-endothelial cells of the hosts. Further, unpigmented schizonts of *P. vivax*, *P. falciparum* and *P. malariae* have been found in the brain and bone marrow of man and exo-erythrocytic schizonts of *P. gonderi* in monkeys. The type of host cell in which schizogony occurs, is, perhaps, a biological reflex of the interrelation of the host and the parasite, and differences in behaviour may be indications of different degrees of adaptation of each species of *Plasmodium* and *Haemoproteus* to its respective host. Corradetti (1938) has reunited the

Plasmodiidae and Haemoproteidae into one group, Plasmodiidae Mesnil, 1903, as both groups of organisms have schizogonic stages in tissue cells. As the full life cycles of many members of both families have not yet been ascertained, the merging of them at present would probably cause more confusion and inconvenience than retaining them, and for these reasons the generic names *Plasmodium* and *Haemoproteus* are used herein. The placing of amphibian Haemosporidia in these genera is based on morphological details and the stages of development found in the vertebrate host.

The genus *Leucocytozoon* also is retained. These parasites may be leucocyte-inhabiting haemogregarines, but their habitual amazing ability to live and multiply in cells that are usually parasite-destroying seems to indicate differentiation from the erythrocytic haemogregarine.

Lankesterella canadensis n.sp. (Pl. III, figs. 1–8)

An interesting new species of *Lankesterella* has been observed in the blood of two *Rana catesbiana* out of approximately three hundred examined by us over a period of five years. One of the infected frogs was captured in a pond near Montreal, the other was caught in Lake Manitou. Both were slightly infected. Neither had any ectoparasites. The morphology of the *Lankesterella* was the same in each frog.

The intracorpuscular forms or trophozoites (Figs. 1, 2), assumed to be derived from sporozoites, are mostly crescentic to bean-shaped, with deeply staining cytoplasm. At each pole of the organism a large vacuole is present. The nucleus is distinct, varying somewhat in appearance, being sometimes granular (Fig. 2), but occasionally forms with a karyosome and chromatin granules on the nuclear membrane have been seen (Fig. 1). Much variation in size is shown, the organisms ranging in length from 10·6 to 19·2 μ and in breadth from 3 to 7·8 μ. Parasites from heart blood, lung and kidney are usually smaller than those from the spleen and liver of our specimens.

Extracorpuscular vermicules (Figs. 3, 4) have also been observed. In life these move actively, extending and contracting as they progress among the erythrocytes. When quiescent, they resemble the intracorpuscular forms and the polar vacuoles are noticeable. Extremely few encapsuled parasites, free in the plasma, have been observed, the thin capsule staining red with Giemsa (Fig. 5). No pigment granules have been seen in any of these forms.

No multiplicative stages have been detected in erythrocytes, whether in blood or organ smears. But, in extremely few endothelial cells of the liver of both *Rana catesbiana* and in the kidney of one, coccidia-like stages have been seen (Figs. 6, 7). These are often close to or partly enclosing the nucleus of the endothelial cell (Fig. 6). The cytoplasm is fairly deeply staining and from 12 to 30 nuclei are present. In some, there is segregation of the cytoplasm and distinct, small vermicular merozoites have been seen, together with free nuclei (Fig. 6), while in other cases the merozoites have been well formed and separated from one another within the endothelial cell (Fig. 7). No cyst wall has been seen, so that these bodies are schizonts and merozoites. The merozoites are 3·3–4·8 by 1·1–1·8 μ.

The remarkable gametogonic stages described by Nöller (1920) we have not had the good fortune to observe, with the exception of some oval, multinucleate masses enclosed in thin cysts, which appear to be young segmenting oocysts (Fig. 8). In one case, adjacent to the oocyst, was a small crescentic *Lankesterella*, the two vacuoles of which

were very obvious. Oocysts with fully formed sporozoites have not been found by us, multinucleate oval cysts up to 21 by 16μ, that is, relatively young ones, only having been present in our sparsely infected material. Intensive search of both bullfrogs for *Isospora*, *Eimeria* or other coccidian to which the apparent oocysts might be related was entirely negative. The association of cysts with young *Lankesterella* is suggestive of relationship.

The genus *Lankesterella*, according to the researches of Nöller (1912, 1913, 1913*a*, 1920), has a life history of the coccidial type, schizogony and gametogony occurring in endothelial cells of the internal organs of the frog and vermicular forms, apparently derived from sporozoites, being injected into frog tadpoles by leeches. Nöller (1912, 1913, 1913*a*) observed macro- and microgametocytes in endothelial cells and in 1920 described anisogamy, followed by oocyst formation with many sporozoites. The trophozoites or intracorpuscular vermicules, developed from sporozoites, were distinguished in *L. minima* by Nöller by the possession of two vacuoles, one on either side of the nucleus. The leech, *Hemiclepsis marginata* (= *Placobdella marginata*), was the transmitting agent, injecting sporozoites of *Lankesterella minima* from the blood plasma into tadpoles.

The complete life history of the *Lankesterella* from *Rana catesbiana* has not been worked out. We have not observed macro- and microgametocytes with certainty, and we do not know the transmitter. The parasite belongs to *Lankesterella*, as intracorpuscular forms with two vacuoles occur, schizogony and merozoite formation have been found in endothelial cells of internal organs, and oocysts also have been found therein. This *Lankesterella* differs from *L. minima*, the type and only species, in the dimensions of the intra-erythrocytic stages being much larger, the two vacuoles of these stages being at the poles and not near the nucleus, and in details of nuclear structure. As far as we can ascertain, *Lankesterella* has not been recorded previously either from *Rana catesbiana* or from Canada. Kudo (1922) stated that he had not observed it in frogs from New York or Illinois, both much farther south, and Illinois very far west of Montreal; nor have we found other reference to it in the United States. From its morphology, its host and its locality it is distinct from *Lankesterella minima* and is considered to be a new species, for which the name *Lankesterella canadensis* n.sp. is proposed, with characters as herein outlined.

Haemogregarina myoxocephali n.sp. (Pl. III, figs. 9–14)

A haemogregarine has been found in the blood of the longhorn sculpin, *Myoxocephalus octodecimspinosus*. One out of five caught off Halifax and one out of five from St Andrews were infected. No ectoparasites were present on the fish.

The haemogregarines, with rare exceptions, are intra-erythrocytic. They vary somewhat in shape, the majority being rather sausage-like (Figs. 9, 11, 12), a few crescentic (Fig. 10) and very few recurved (Fig. 13). The finely granular cytoplasm of the intracorpuscular forms stains well. The nucleus is always displaced towards one pole, which it often occupies (Figs. 9, 12, 13), and is deep-staining, with relatively large, coarse chromatin granules. The unthickened nuclear membrane is distinct. The haemogregarines are 5·5–9·2 by 2·2–2·9μ, their nuclei being 1·5–4·6 by 1·5–2·9μ. Intracorpuscular vermicular forms were few (Fig. 13). They varied from 8·1–12·6 by 1·5–2·2μ, with nuclei 2·6–4·4μ, their breadth being that of the body. Free vermicules (Fig. 14) were rare; they were 5·9–11·1 by 1·5–2·2μ. No parasites with a capsule or cytocyst have been found.

The haemogregarines from the St Andrews' fish are slightly larger than those from the Halifax one, but much overlapping of dimensions occurs. There are no other differences.

In both hosts the infected erythrocytes are enlarged, with displaced and usually reduced nuclei, but in some fresh preparations the nuclei of infected erythrocytes were pale and seemed bladder-like.

Regarding the identity of this haemogregarine, careful comparison has been made with illustrations of fish haemogregarines from all parts of the world, but none are quite identical with this one from *M. octodecimspinosus*. Most piscine haemogregarines are much larger and have less well-defined nuclei. Henry (1912) has described *Haemogregarina anarhichadis* from *Anarhichas lupus*[1] from off the Shetland Isles, the small forms of which show some resemblance to the one from *Myoxocephalus*, but the Canadian organism is much smaller, is far less polymorphic, and has a different nuclear structure, while there is no development of polar 'purple-staining substances', characteristic of *Haemogregarina anarhichadis*. The two are not identical. From literature, we have found no record of a haemogregarine from the longhorn sculpin. It is considered to be a new species in a new host, and is named *Haemogregarina myoxocephali* n.sp., with characters as here outlined.

Haemogregarina urophysis n.sp. (Pl. III, figs. 15–18)

A haemogregarine has been observed in blood and organ smears of one out of four freshly caught white hake, *Urophysis tenuis*, from Halifax, Nova Scotia, and in one out of five from St Andrews, New Brunswick. The Halifax fish was taken in August, two years after the one from St Andrews. A moderately heavy infection was present in each fish, though they seemed in excellent condition. Neither had ectoparasites.

This haemogregarine is always intra-erythrocytic and neither in blood nor organ smears have free vermicules been found. Almost all the parasites are bean-shaped or reniform, with a well-marked curvature, often in contact with the nucleus of the erythrocyte (Figs. 15–17). The cytoplasm is usually distinctly alveolar (Figs. 15–17). The nucleus is large, often roughly median (Figs. 15, 16), sometimes somewhat nearer one pole (Fig. 17); it stains moderately well. The chromatin in most is evenly distributed as fine granules (Figs. 15, 16); exceptionally, larger chromatin granules occur on the nuclear membrane (Fig. 17). The reniform haemogregarines from the Halifax fish are 11·8–14·8 by 3–4·4 μ, their nuclei being 5·9–8·2 by 2·6–3·7 μ, while those from the St Andrews' specimen are 11·1–14·4 by 3·3–5·2 μ, with nuclei 5·2–8·2 by 3–4·4 μ, the differences in range being negligible and the morphology the same. The very small number of intra-erythrocytic vermicules (Fig. 18) were 12·2–17·8 by 3–3·5 μ, and the reflexion was slight.

The infected erythrocytes are much hypertrophied, their nuclei are reduced in size, and both nuclei and cytoplasm stain less than normal cells.

The nearest affinity of the haemogregarine of *Urophysis tenuis* appears to be *Haemogregarina aeglifini* Henry (1913), which occurs in haddock (*Gadus = Melanogrammus aeglifinis*) from fishing grounds north of Scotland. *H. aeglifini* is polymorphic. Small, slightly bent, and large reniform haemogregarines have numerous basophilic granules in their cytoplasm, while their nuclei are composed of very large chromatin granules 'bunched together'. The intermediate type have a pale globular body present, as well as

[1] The Official List of Zoological names (1926) (*Proc. Biol. Soc. Wash.* **39**, 100) gives the genus as *Anarhichas*, not *Anarrhichas*; presumably the specific name of the haemogregarine must be amended to *anarhichadis*. We much regret we have been unable to get in touch with the author.

basophilic granules. While the dimensions of the large form of *H. aeglifini* are much the same in range as those of the parasite of the white hake, the latter can be separated by its alveolar cytoplasm without basophilic granules and its nuclear structure. We have examined the blood of a number of haddock in Canada, but have found neither *H. aeglifini* nor the haemogregarine of *Urophysis* therein. The latter appears to be distinct and is named *Haemogregarina urophysis* n.sp., with characters as herein outlined.

Other haemogregarines of Amphibia and fishes

Many haemogregarines sens lat. from frogs and toads in different parts of the world have been recorded, and it has rarely happened that the discoverers have had the good fortune to elucidate their life histories. We are in the same position, and here describe the morphology of two other amphibian haemogregarines that seem fairly distinct from the species described by Kudo (1922) from *Rana clamitans* and *R. pipiens* from New York and Illinois, and record three haemogregarines from Canadian fishes.

Haemogregarina sp.

Other amphibian haemogregarines

This haemogregarine has been found in the blood of *Rana pipiens* captured at Montreal, Montreal West, Lachine, Lac Gaumont and Labelle City, and in the blood of *Bufo americanus* from Lachine. Over a period of five years, more than two hundred *Rana pipiens* have been examined, of which two harboured heavy and five slight infections. No ectoparasites and no sanguivorous insects were associated with the infected hosts.

The haemogregarines occur in erythrocytes or as free vermicules in the plasma. Organ smears showed only the same stages as the blood. The intracorpuscular forms are elongate vermicules, lying to one side of the host cell nucleus but never surrounding it. Both ends of the organism are rounded, but one end is usually much broader than the other, which tapers. A small number are banana-like in outline. The cytoplasm is finely granular, without vacuoles, pigment or other inclusions. The nucleus is distinct, with a delicate nuclear membrane enclosing fine chromatin granules, which in some are uniformly distributed, but occasionally are clustered in bands or streaks. In a few of the smallest forms only was the nucleus karyosomatic. In elongate extended organisms the nucleus occupies the whole width of the body; in contracted ones it occupies part of the width. The intracorpuscular forms are 6·6–16·6 by 1·8–4·4 μ, their nuclei being 1·6–5·2 by 1·6–4·4 μ. Double infections and multiplicative stages of the parasite have not been observed.

The erythrocytes of *Rana pipiens* and *Bufo americanus* showed cytoplasmic hypertrophy, varying degrees of nuclear displacement and general decrease of stainability.

This haemogregarine differs from the form described by Kudo (1922) from *Rana clamitans* and *R. pipiens* in dimensions, constant absence of a capsule or cytocyst, and absence of refractile granules in the cytoplasm. It has not been found in *R. clamitans* in Quebec Province.

A sparse infection with a large haemogregarine has been observed in the blood of a large male *Rana catesbiana* caught at Lake Manitou. All the parasites were at the same stage of development, were intra-erythrocytic, whether in blood or organ smears, and the infected erythrocytes were greatly enlarged.

The haemogregarines are large vermicules, with bluntly rounded ends, curved round

the periphery of much of the host cell, and displacing its nucleus to one side. Each has almost homogeneous cytoplasm, a small nucleus with unthickened nuclear membrane and contents staining uniformly with Giemsa stain. Almost all the organisms were about 50μ in length and 2·2–3·3μ in breadth, their nuclei being about 3 by 2·2μ.

R. clamitans and *R. pipiens* collected at the same time from Lake Manitou were negative for haemogregarines.

Other haemogregarines of fishes

Haemogregarina aeglifini Henry has been found in *Gadus callarias* from off Labrador, the host and locality being new.

Haemogregarina platessae Lebailly, or a form closely allied thereto, is recorded from the Canadian flounder, *Pseudopleuronectes americanus*.

A haemogregarine, probably *Haemogregarina bigemina* Laveran & Mesnil, has been observed in the blood of the eel pout, *Zoarces angularis*, obtained in Montreal. Bentham, quoted by Reichenow (1932), found *Haemogregarina bigemina* in *Zoarces viviparus* in Europe.

Plasmodium catesbiana n.sp. (Pl. III, figs. 20–25)

A number of bullfrogs, *Rana catesbiana*, have been examined by us from time to time, from the neighbourhood of Old Mill (Back River), Montreal. In two out of one collection of six bullfrogs, a very interesting, small haematozoon has been observed. Unfortunately, the infection was not heavy, and, though over seventy more *R. catesbiana* have since been examined from the same area, the organism has not been found again. Erythrocytes only have been found infected.

The youngest parasites observed are probably derived from recently penetrated sporozoites or merozoites. They are small, oval bodies, many being about 3 by 2·2μ. The cytoplasm is finely granular, the nucleus rounded and karyosomatic (Fig. 20). Larger forms are amoeboid in life and irregular in outline (Fig. 21), while others much resemble the 'signet ring' stage of human *Plasmodium* (Fig. 22), being rounded, with a clear vacuole. The nucleus is karyosomatic. Some of these small forms contained one or more pigment granules, these being yellowish brown in life. These 'rings' varied a little in size, the largest being about 6·6μ in diameter. Co-existing with the foregoing, schizonts have been observed. These are rounded to oval, the largest ones reaching 7·2 by 6·2μ in diameter. Stages of nuclear division into four, six and eight merozoites have been found, the merozoites, clustered around the rather coarse pigment, forming a rosette (Fig. 23). The same blood smears contain forms that may be interpreted as gametocytes (Figs. 24, 25). They differ from the trophozoites in having denser cytoplasm, no vacuole and nuclei with slightly more prominent karyosomes. Slight indications of dimorphism occur, some, having denser, more deeply staining cytoplasm may be macrogametocytes (Fig. 24), others with clearer, pale-staining cytoplasm microgametocytes (Fig. 25).

Examination of organ smears and sections revealed no trace of any other stages of the organism. A species of *Gerris*, collected from Old Mill, had no stages of the parasite either in the salivary glands or in or on the alimentary canal.

The effect of the parasite on the host cell is out of all proportion to its size. The erythrocyte is enlarged, its nucleus becomes enormously hypertrophied, and the cytoplasm reduced (Fig. 22). The stainability of both nucleus and cytoplasm of infected

erythrocytes is poor, and parasites situated above the nucleus could be readily distinguished. In fresh preparations balloon-like structures indicated infected erythrocytes.

The assigning of some Haematozoa found in Amphibia to the genus *Plasmodium* is based on their morphology and developmental stages in the blood of their hosts. The occurrence of *Plasmodium* in Reptilia has been generally recognized on these grounds, but there seems to have been some reluctance to assign parasites of other cold-blooded hosts to this genus. However, the systematic position of this haematozoon appears to be within the genus *Plasmodium*. It is pigmented, has signet-ring-like trophozoites, rounded schizonts forming eight merozoites, and probable rounded macro- and microgametocytes, all occurring in erythrocytes. Morphologically, it is unlike any of the pigmented *Plasmodium* from cold-blooded vertebrates such as snakes and tortoises. It is considered to be a new species and is named *Plasmodium catesbiana* n.sp., with characters as herein outlined.

Plasmodium bufonis n.sp. (Pl. III, figs. 26–30)

A second species of amphibian *Plasmodium* has been found in the blood and organ smears of one out of five *Bufo americanus* caught at Laval des Rapides. The parasites occur only in erythrocytes in the peripheral blood, heart blood and liver blood; preparations of lung, kidney, spleen and intestinal tissues contained no parasites.

Young forms or trophozoites, with alveolar cytoplasm, pigment and a small vacuole, have been seen in small numbers.

Schizogony of this *Plasmodium* takes place in erythrocytes, especially those in blood smears of the heart and liver. A series of stages of division have been observed. The large schizonts are oval. The earliest stage shows two nuclei. Other divisions result in four and eight nuclei, and in some the chromatin masses had not fully separated or were undergoing further division (Figs. 26, 27). Later, the nuclei became elongate and arranged en barillet and cytoplasmic segregation into eight merozoites began (Fig. 28). The pigment of the schizont usually occupies one pole of the organism (Figs. 26–28). Exceptionally, schizonts with four merozoites only have been observed. Perhaps micro- and macromerozoites are ultimately produced, but we have not seen them.

Two forms of apparent intra-erythrocytic gametocytes have been found. Some are oval or slightly reniform (Fig. 29) and may be macrogametocytes; others are vermicular and bent on themselves (Fig. 30) and may be microgametocytes. Both have somewhat pale-staining cytoplasm, that of the oval forms being denser and more deeply staining. Pigment granules are present. The nuclei are rounded, with small chromatin granules that stain well. The oval forms are 10·5–16 by 5·2–8·3 μ. The vermicular forms are 18·6–27·3 by 4·1–5·3 μ. Some workers have suggested that oval forms are produced by fusion of the limbs of the vermicules. Though many hours were spent by us in watching the living organisms, no fusion of limbs was ever observed. In some erythrocytes a clear area often surrounds the parasite, and in exceptionally few cases a capsule or cytocyst has been observed. These encapsuled forms very occasionally have been seen free in the blood plasma.

No ectoparasites were present on the infected toad. Many mosquitoes from the locality were dissected but were negative for *Plasmodium*, so that there is no information regarding the life cycle in the invertebrate host.

This interesting organism morphologically is a species of *Plasmodium*. Its dimorphic

gametocytes, large oval schizonts with schizogony into four and eight merozoites, are distinctive. The pigmented *Plasmodium* from *Bufo americanus* from Laval des Rapides seems unique. It is, therefore, named *Plasmodium bufonis* n.sp., with characters as herein set forth.

Haemosporidian from **Centropristis striatus** (Pl. III, fig. 19)

A haemosporidian has been observed in the erythrocytes of a black sea bass, *Centropristis striatus*, bought in Montreal. It presents several interesting features.

The parasites are almost invariably intra-erythrocytic. Almost always two are present in an erythrocyte. Sometimes they are apposed, sometimes separate from one another. They are elongate, reniform or conical in outline (Fig. 19, left and right), with coarsely granular cytoplasm and oval granular nuclei. The conical forms stain slightly paler than the reniform ones. The organisms are much about the same size, 8·5–10·4 by 1·5–2·2 μ. A very few reniform vermicules, 8·5–9·3 μ, have been found free in the plasma. Unless the slight differences in shape and staining are considered sufficient criteria, there is no certain sexual dimorphism. Multiplicative stages have not been found, organ smears showing only intra-erythrocytic parasites.

Laveran & Mesnil (1901) described *Haemogregarina bigemina* from *Blennius pholis* and *B. montagui* from the French coast. Lebailly (1906) repeated their description and confirmed it. Two vermicular haemogregarines occur in each erythrocyte, often close to and curved parallel to each other. Stages of binary fission into two and, in one case only the beginning of division into four, were observed. No such schizogony has been found in the parasite of the Canadian *Centropristis*, the morphology is different and the dimensions are smaller than those of *Haemogregarina bigemina*. If the constant occurrence of two parasites within one erythrocyte were due to fission, it would suggest development of the *Plasmodium* type, but the slight differences in shape might also be indicative of gametocytes. Further work is necessary before any conclusion can be attained.

Haemoproteus laurentiae n.sp. (Pl. III, figs. 31–36)

This *Haemoproteus* has been obtained in the blood of one out of three *Bufo americanus* from the bank of the St Lawrence at Montreal South, and in two out of seven *B. americanus* collected on one afternoon from the banks of Lake Manitou. Nine toads collected two years later at the same sites were negative.

Most of the parasites are intracorpuscular. The smaller forms are intracorpuscular, reniform or bean-shaped vermicules, with homogeneous cytoplasm and deep-staining, relatively large, nuclei, in which the chromatin is compact or somewhat stranded (Fig. 31). In some, pigment granules or rodlets are present. These smaller forms are 7·6–10·6 by 3–3·8 μ. Larger, more or less oval forms, considered to be gametocytes (Figs. 32, 34), usually intra-erythrocytic, have homogeneous cytoplasm and densely granular nuclei. The rod-like or bacilliform granules of pigment are often distributed in a ring round the nucleus (Fig. 32) or partly so (Fig. 33), and, very exceptionally, in addition to the juxta-nuclear granules, a few separate bacilliform granules have been seen at one pole of the organism (Fig. 34). Very occasionally, an oval form with characteristic pigment has been found with a cytocyst (Fig. 33), rarely intra-erythrocytic, usually free in the plasma. These oval forms mostly range from 10·1–17·7 by 3–7·2 μ. Intra-erythrocytic, looped vermicules or gametocytes (Fig. 35) have been seen, the limbs being reflexed on each

other and never embracing the nucleus of the host cell. They, too, have rod-like pigment. The nucleus of these vermicules is compact and rounded to oval, varying somewhat in shape when it is situated near the point of flexion of the organism. Free vermicules (Fig. 36) have been found in small numbers. Intracorpuscular vermicules are 21·5–23·3 by 3·7–4·6 μ, while free forms are 20·7–25·7 by 3·7–5·2 μ. Multiplicative stages have not been observed either in blood or organ smears.

The infected erythrocytes are enlarged, their nuclei displaced away from the parasites and often deformed.

The presence of distributed rod-like pigment granules seems to be the special character of this *Haemoproteus*. We have made comparison with *Haemoproteus* from other cold-blooded hosts such as Amphibia and Reptilia, but have not found any organism corresponding exactly with this form. It appears to be new to science. Provisionally, it is named *Haemoproteus laurentiae* n.sp. (the species' name being arbitrarily derived from one site of the host), with characters as herein outlined.

Haemoproteus lavalia n.sp. (Pl. III, figs. 37–40)

This *Haemoproteus*, designated *H. lavalia* n.sp., has been found in one out of five *Bufo americanus* caught at Laval des Rapides.

All the *Haemoproteus* observed have been intra-erythrocytic and are probably gametocytes. They are oval to slightly pyriform in outline and 10·8–16·2 by 5·2–7·8 μ in diameters. The cytoplasm is finely granular and stains fairly deeply. The pigment is restricted to curved bands (Figs. 37–40), usually curving roughly parallel to the edge of body (Figs. 37, 38) and occasionally with one end of the pigment zone passing on to the nucleus (Fig. 39) or outwards to the periphery (Fig. 40). The pigment consists of rounded to somewhat angular coarse granules. The nuclei are usually nearer one pole of the organisms. They are rounded to oval, with chromatin in fairly uniformly disposed granules, and are 3·9–6·2 by 3–4·5 μ. Most of the *Haemoproteus* are in contact with the cytoplasm of the host erythrocyte; a few are surrounded by a clear zone (Fig. 39) and, in exceptionally few instances, a cytocyst has enclosed them (Fig. 40). No evidence of sexual dimorphism, other than hardly significant differences in cytoplasmic staining, has been found. Multiplicative stages have not been observed either in blood or organ smears, except that in lung smears, in addition to intra-erythrocytic parasites, uninucleate forms identical with the latter, have been seen in extremely few endothelial cells and may be potential schizonts, but no definite evidence of schizogony was obtained.

The infected erythrocytes are much enlarged, their nuclei displaced and often reduced in size and their cytoplasm stains palely.

Having examined illustrations of many *Haemoproteus* and haemogregarines sens lat., no amphibian form with pigment in constant, well-defined bands has been found. Examination of many smears of amphibian blood made in South Africa has also shown no such parasite. *Bufo americanus* also appears to be a new host. The organism, then, seems to be here described for the first time, and is named *Haemoproteus lavalia* n.sp., with characters as herein set forth.

Haemoproteus lanoraiea n.sp. (Pl. III, figs. 41–45)

A distinctive *Haemoproteus* has been found in blood and organ smears of one out of three *Bufo americanus* caught at Lanoraie, P. Que. The number of parasites in the circulating lung and heart blood was relatively small, few occurred in the liver and kidneys,

extremely few in the spleen, and none in preparations of the bone marrow and intestinal wall. The parasites were most numerous in lung preparations, and in these, in addition to intra-erythrocytic forms, a few were found within endothelial cells, but, unfortunately, were at the same stage of development as those in the erythrocytes.

The intra-erythrocytic parasites or gametocytes are of two types. The majority are compact, oval to slightly conical in outline (Figs. 41–43). They have hyaline cytoplasm and, exceptionally, a few larger cytoplasmic granules may be present. The nucleus is round to oval in outline and consists of distinct, small, fairly deeply staining granules. The nuclear membrane is often indistinct. In a few cases a capsule is present (Fig. 42). These forms are 11·8–16·7 by 4·4–8·5 μ, with nuclei 3–5·2 by 2·2–3·7 μ. The second type of gametocyte has a tadpole-like appearance (Fig. 44), with a narrow recurved portion parallel to the wider one. They measure 20–23·7 by 4·1–4·8 μ, and their nuclei are 3–4·1 by 2·2–3·7 μ. Some encapsuled forms have been found free in the plasma (Fig. 44). The pigment of both types of gametocytes consists of a mass of small refractile granules, not easy to detect in life, very pale yellow to light brown in colour, densely crowded at one pole of the organism, and giving the appearance of a compact, granular cap, which stains almost as densely as the nucleus of the host cell with iron haematoxylin, Delafield or Leishman stain, the effect being striking (Figs. 41–45).

Some oval parasites have been found in endothelial cells of the lung (Fig. 45), but schizogony has not been detected.

No ectoparasites were present on any of the toads. The further stages of development are unknown.

The effect of the *Haemoproteus* on the host erythrocytes is marked. The nuclei show obvious displacement, often to one side (Figs. 41, 42), but occasionally even transverse to the parasite (Fig. 43). Reduction in size of erythrocyte nuclei has been observed, and in a few cases they have entirely disappeared.

This parasite belongs to the genus *Haemoproteus*, gametocytes occurring in the blood and schizogony probably in the lungs. The remarkable caps of pigment granules seem not to have been previously described for amphibian Haematozoa, though rather different forms of them have been reported from fish, in which, however, discrete pigment granules cannot be distinguished. Minchin & Woodcock (1910) gave an account of the development of deep-staining substances in the cytoplasm of *Haemogregarina rovignensis* from the gurnard, *Trigla lineata*, and Henry (1912) described a similar development in *Haemogregarina anarhichadis* from the catfish, *Anarhichas lupus*. The caps of pigment granules seem to differentiate the *Haemoproteus* of *Bufo americanus* from other species. It is considered to be a new species and is designated *Haemoproteus lanoraiea* n.sp., with characters as outlined herein.

Leucocytozoon salvelini n.sp. (Pl. IV, figs. 1–7)

A new *Leucocytozoon* has been found in the polymorphonuclear and more rarely mononuclear leucocytes of three speckled trout, *Salvelinus fontinalis*, caught in a lake in the Gaspé Peninsula, Quebec. The fish were apparently in good condition, their blood and organs harboured no other Protozoa, nor were ectoparasites present on either their skin or gills.

In fresh hanging-drop preparations the young vermicules move backwards and forwards in the host cell, displacing the nucleus and, at times, causing prolongations of the

cytoplasm of the leucocyte at the poles, comparable with what has been seen by one of us (H. B. F. 1910) in *Leucocytozoon lovati* of grouse and in *L. anatis* of Canadian ducks.

The young intraleucocytic parasites (Fig. 1) are about 8·5 by 1·5–2 μ, somewhat curved or banana-shaped, with clear cytoplasm and distinct nuclei, with dense staining granules. Larger vermicular trophic forms (Figs. 2, 4) often have one end broader than the other. They are from 11·6–18·9 by 1·8–3·7 μ. A few oval intraleucocytic forms (Fig. 3) have been observed. They and the larger vermicular forms have large nuclei. Occasionally vermicules escape into the blood plasma and can be seen free therein (Fig. 5). Encapsuled parasites have not been observed.

Intraleucocytic parasites, that are probably gametocytes, have been observed in small numbers. Some are spherical, pale-staining, with a distinct nucleus and karyosome (Fig. 6). These may be microgametocytes. Others are larger, with more densely staining cytoplasm and nucleus of fine chromatin granules (Fig. 7). These may be macrogametocytes. Further development is not known.

The infected polymorphonuclear leucocytes became reduced in size, but the remains of their nuclei could always be seen.

This organism is placed in the genus *Leucocytozoon*. Many have been described from birds. However, Yakimoff (1917) found one in an undetermined Russian fish and named it *L. nina-kohl-yakimovi*, and Henry (1913) reported one from *Gadus aeglifinis*. These are the only fish *Leucocytozoa* of which we have found records. Whether the host be fish or bird, the *Leucocytozoon* is highly specialized physiologically, as already noted, and is adapted to extremes of conditions of life in cold- and warm-blooded vertebrates. Unfortunately, Yakimoff's 1917 paper does not contain a full description of his fish *Leucocytozoon*, and apparently no further account of it has been given. The parasite of *Salvelinus fontinalis* is the third to be recorded from fish; it parasitizes only the leucocytes; trophic forms and gametocytes were present at the same time in the fish, which had no other Haematozoa. The organism seems new to science and is named *Leucocytozoon salvelini* n.sp., with characters as herein outlined.

Dactylosoma salvelini n.sp. (Pl. IV, figs. 8–16)

A new *Dactylosoma* has been observed in one speckled trout, *Salvelinus fontinalis*, out of several hundreds that have been examined by us from many localities in the Province of Quebec. The infected fish was caught by a friend in his private stream in the Laurentians and was apparently in splendid condition. Blood and organ smears showed a sparse infection of the erythrocytes with a *Dactylosoma*.

The youngest stage observed is a small, oval to round body, with a round, karyosomatic nucleus. These are trophozoites (Fig. 8). Schizonts are more numerous. Binucleate forms (Fig. 9) have almost homogeneous cytoplasm. They are small, many being about 3 by 2·2 μ. Tetranucleate schizonts (Fig. 10) and schizonts with six, seven and eight nuclei have been found (Figs. 11–13). Most of the mature schizonts are fan- to wedge-shaped (Figs. 12, 13) with the nuclei arranged on two sides or peripherally. In some, cytoplasmic segmentation to form merozoites is indicated (Fig. 13), in others merozoites have formed and, in a few cases, the merozoites have separated completely and are scattered in the cytoplasm of the erythrocyte (Fig. 14). The range of dimensions of tetra- to octonucleate schizonts varies from 5·8–8·5 by 3·7–7 μ, the largest dimensions being exceptional.

Two types of gametocytes have been observed but are not markedly different in shape. One is oval to reniform, with rather granular cytoplasm and a rounded nucleus with rather dense chromatin (Fig. 15). The second type is also reniform, has much paler staining cytoplasm and a nucleus of scattered chromatin granules (Fig. 16). The two forms overlap in dimensions and are from 4·4–7·8 by 1·5–3 μ. Nöller considered the smaller vermicular forms of *D. ranarum*, with small karyosomes and homogeneous cytoplasm, to be microgametocytes, and forms with larger karyosomes and vacuolated cytoplasm to be macrogametocytes. The *Dactylosoma* of *Salvelinus fontinalis* has no such clear-cut distinction between its gametocytes. From comparison with other Haemosporidia and their staining reactions, we are inclined to consider the forms with granular cytoplasm and denser chromatin as macrogametocytes and those with paler staining cytoplasm and granular nuclei as microgametocytes. Further stages in development have not been found.

No ectoparasites occurred on the trout and no leeches could be found in the neighbourhood. Dissection of various aquatic Hemiptera revealed no stages of *Dactylosoma*.

Infected erythrocytes stained slightly less deeply than normal ones. Enlargement of them, with nuclear displacement, often occurred at the late schizont stage (Figs. 11, 13).

With regard to the identity of the *Dactylosoma* of *Salvelinus fontinalis*, as far as we can ascertain from search of literature, only one species of *Dactylosoma* is known from fish. This is *D. mariae*, described and fully illustrated by Hoare (1930) from four species of *Haplochromus*, the fish having been taken from Lake Victoria Nyanza, near Entebbe, Uganda. The parasite of the trout cannot be *Dactylosoma mariae*, for this is characterized by producing 'never more and never less' than four merozoites from a schizont, these being arranged in a rosette or cruciform manner. The presumed gametocytes also differ in morphology, while the dimensions of the trout parasite are smaller than those of *D. mariae*.

The possibility of the trout *Dactylosoma* being related to one parasitic in Amphibia has been considered. In Europe, Labbé (1899) described *Laverania* (= *Dactylosoma*) *splendens* from European frogs, one form being elongate finger-like, the other lobed and amoeboid, while 5–12 merozoites were formed around a residual body. Nöller (1913, 1913*a*) described *Dactylosoma splendens* in detail. Schizonts were 4–9 μ, the number of merozoites 4–16. A second type of schizogony produced merozoites which developed into gametocytes. Comparing our trout *Dactylosoma* with that from European frogs, one type only of schizogony has been observed, the number of merozoites is eight and the dimensions of all stages are different.

Dactylosoma occurs in African frogs and toads. Dutton, Todd & Tobey (1907) found it in three species of *Rana*, in *Rappia marmorata* and *Bufo regularis* in the Congo. Their slender vermicules were 13–15 by 1·1 μ, had a nucleus with eight chromatin masses, penetrated erythrocytes and became schizonts which formed fanwise rosettes of 10–16 merozoites. We also have examined *Dactylosoma* in the blood of *Bufo regularis* in the Transvaal, but eight merozoites only were formed, and, like the Congo form, the parasite was much larger and more robust generally than the delicate trout parasite.

Mathis & Léger (1911) have described *Dactylosoma splendens* from *Rana guntheri* in Tonkin. This differs from the European and African forms. The youngest schizonts of the *Dactylosoma* of the Tonkin frog are about twice the size of those of the trout, and other dimensions differ correspondingly. Also, its merozoites are very symmetrically arranged.

It is clear that the *Dactylosoma* of the trout cannot be assimilated with *D. ranarum* from European, African or Asian frogs.

On the American continent, Durham has recorded a *Drepanidium* (now *Dactylosoma*) from a Brazilian toad. Kudo (1922) does not mention *Dactylosoma*. In the succeeding section we are describing a *Dactylosoma* from *Rana sylvatica* in Quebec, which differs from *Dactylosoma ranarum* from other parts of the world and is much larger than the trout *Dactylosoma*.

Two species of *Dactylosoma* have been described from Urodeles. Fantham (1905) described a haematozoon from the European newt, *Triton cristatus*, under the name of *Lankesterella tritonis*. The discovery by Nöller of endothelial schizogony of *Lankesterella* has caused its transfer to *Dactylosoma*. It is the smallest species of *Dactylosoma* known, vermiform stages being 5–6 by 1–1·5 μ, while the schizonts producing 6–9 spherical merozoites are only 2·5–3·5 μ in diameter. It obviously cannot be the same as the parasite of *Salvelinus fontinalis*. Nigrelli (1929) has described *Dactylosoma jahni* from the spotted newt, *Triturus viridescens*, in Pennsylvania. The morphology and dimensions at once separate it both from *Dactylosoma tritonis* and from the trout parasite. Further, it produces but four merozoites, which recalls the four of *D. mariae* in African fish, though the morphology is different.

From the foregoing, the *Dactylosoma* from Canadian *Salvelinus fontinalis* does not agree in morphology or development with *Dactylosoma* either from Anura, Urodela or the one from African fish. It appears to be a new species, parasitic in a new host and new locality. It is designated *Dactylosoma salvelini* n.sp., with characters as herein outlined.

Dactylosoma sylvatica n.sp. (Pl. IV, figs. 17–35)

An interesting *Dactylosoma* has been found in the blood of one adult male and one adult female *Rana sylvatica* out of seven frogs collected from the wooded banks of a stream near Quebec City. The male frog was more heavily infected than the female. No ectoparasites occurred on them. Examination of species of *Gerris* and *Hydrometra*, the only insects obtained by netting and sweeping, gave no evidence of them being transmitters of the *Dactylosoma*.

The youngest trophozoites observed (Fig. 17) are small, slightly amoeboid organisms, many being about 4·4 by 3 μ in diameters. The cytoplasm is homogeneous, the nucleus rounded, with a distinct karyosome. Larger trophozoites (Fig. 18) have alveolar cytoplasm and range from 7–8·5 by 6·3–7·6 μ. No cytoplasmic inclusions have been observed.

The smallest schizonts are binucleate (Fig. 19). Forms with four nuclei (Fig. 20) are often about 5·2 by 4 μ. Oval schizonts with eight nuclei vary from 5·2–10·7 by 3·7–5·9 μ. The nuclei early arrange themselves at the periphery of the parasite (Figs. 21–23), which soon assumes the typical fan- or wedge-shape (Fig. 24). Some small schizonts (Figs. 25, 26) occurred in the male but not in the female frog. Cytoplasmic segregation around each chromatin mass occurs (Fig. 24) and eight merozoites are produced (Figs. 27–29), the clusters measuring 7·4–11·5 by 7–9·3 μ. In a few cases the mass of merozoites has been observed leaving the erythrocyte as a body (Fig. 28). In others, the merozoites break away separately and pass into the plasma as small vermicules (Fig. 30), ranging from 4·4–5·9 by 1·1–2 μ. There is no definite residual body. We obtained no definite evidence of macro- and micromerozoites, despite variation in size among the schizonts.

Vermicular gametocytes occur within erythrocytes and are free in the plasma. Intra-

erythrocytic forms are oval (Fig. 31) to reniform (Figs. 32, 33). Oval forms stain more deeply and have rather denser nuclei. They may be macrogametocytes. The reniform or bean-shaped parasites have rather larger nuclei with distributed chromatin granules and stain faintly. They may be microgametocytes. The intra-erythrocytic forms are 7–12·6 by 1·5–3 μ. Extracorpuscular vermicules (Figs. 34, 35) are 8·1–20 by 1·8–6·6 μ, extreme dimensions being exceptional. Gametocytes with well-marked dimorphic karyosomes, such as Nöller (1912, 1913) described for *D. ranarum*, have not been observed.

In the preceding section on *D. salvelini*, many *Dactylosoma* found in Amphibia have been noted. There is a general similarity between the *Dactylosoma* found in frogs in different parts of the world, though local races or varieties will probably be found to occur. The *Dactylosoma* of *Rana sylvatica* may prove to be such a race or variety, but it differs in dimensions, in details of cytoplasm and nuclear structure and in having one type of schizogony resulting in eight and not sixteen merozoites as in *Dactylosoma splendens*. It obviously differs from *D. jahni* with but four merozoites. It is recorded from a new host, in a new locality. For these reasons it is considered to be a new species and is designated *Dactylosoma sylvatica* n.sp., with characters as herein set forth.

MICROFILARIA

Microfilaria ranae-sylvaticae n.sp. (Pl. IV, figs. 36, 37)

A *Microfilaria* sens lat. has been found in the blood of one *Rana sylvatica* collected near Quebec City. No adult filariid was found either in the gut or in the tissues of the host. The infection appeared to be recent, many of the microfilariae being relatively undeveloped. No ectoparasites were present on the infected frog or on others taken with it.

Examination in vivo was aided by the use of methyl green and of azur II in saline. In life the microfilaria is moderately active, looping into a U shape or, more often, coiling on itself.

The Microfilaria (Figs. 36, 37) is unsheathed. The cuticle is thin. The subcuticular cells are elongate. Numerous somatic cells, not very regularly arranged, are more or less rounded and form the nuclear column. At the rounded anterior end of the body are two large nuclei, the precursors of the mouth apparatus (Figs. 36, 37). Passing posteriorly the following structures have been identified in the more mature larvae: Posterior to the mouth is a clear nerve ring (Fig. 37, *N*). A large, rounded excretory cell (Figs. 36, 37, *Ex.C.*), without a pedicel, communicates with an excretory pore, not easily detected. About half-way down the body is a large 'genital' cell or G_1. About two-thirds down the body two slightly smaller cells, G_2 and G_3, very close together, and still farther posteriorly G_4 can be distinguished. The anal pore (*A.p.*) is still farther posterior and often is seen only with difficulty. The so-called 'Innenkörper' or inner body is only distinguishable as more densely packed nuclei. At the posterior end of the body the nuclear column ends in two rows of two nuclei and two nuclei arranged tandem (Fig. 37). The microfilariae are 55·5–97 by 5·2–10 μ.

The further development could not be traced. Dissection of numerous *Simulium venustum*, a few *Culex pipiens*, some *Gerris* sp. and *Hydrometra* sp. from the locality revealed no microfilariae. No ectoparasites were present on the infected *Rana sylvatica*. It is of interest to recall that Darling (1912), when endeavouring to elucidate the life history of the haemogregarine of *R. marinus* in Panama, found unsheathed microfilariae

in the blood of the toads. The latter were infested with the tick, *Amblyomma varium*, and sections of these ticks showed large numbers of sheathed filariae, larger than those in the blood of the toads. Feng (1931) has found *Anopheles hyrcanus* var. *sinensis* to be the transmitter of *Wuchereria* (*Filaria*) *bancrofti* in Shanghai, proved this experimentally (1936) and has made a detailed study (1933) of *Microfilaria malayi* Brug and *Mf. bancrofti* Cobbold, which, with his illustrations, enables close comparison to be made with microfilariae other than those of man.

The adult nematode parasites of North American frogs from the United States have been described by Walton (1929), and his list of Filariae sens lat. (1935) contains a reference to larval forms of *Filaria columbi* Blanchard from the blood of a toad from Colombia, South America. Walton (1929) has also described adult *Foleyella americana* from the thoracic cavity of *Rana pipiens* and *Foleyella ranae* from *Rana clamitans*. The larval development of *Foleyella ranae* in *Culex fatigans* and that of *Foleyella dolichoptera* in *Culex fatigans*, *C. pipiens* and *Aëdes aegypti* have been elucidated experimentally by Causey (1939), and the same author has made an intensive, comparative, illustrated study of *Microfilaria dolichoptera*, *Mf. brachycoptera* and *Mf. ranae* (1939*a*). We have carefully compared these interesting organisms with those of *Rana sylvatica*, and there are many points of difference in morphology from each of them. The *Microfilaria* from *Rana sylvatica* is nearest in length to *Mf. ranae*, but is much broader. Its nuclear column has more nuclei, less regularly arranged than Causey's three species. The mouth-parts are dissimilar and better defined in the *Microfilaria* of *Rana sylvatica*, in which the 'Innenkörper' is less well defined. Causey was able to assign definite critical positions in terms of percentage of body length to the chief structural features of his larvae. We attempted to do this, but the results were considered inconclusive owing to insufficient material. With regard to the *G* series (often termed 'genital', but not so, by analogy with Feng's developmental results), G_1 is almost median, far separated from G_2 and G_3, which almost touch one another, and G_4 is some distance away, positions not found in any of Causey's species. In consequence, the conclusion is that a fourth species of *Microfilaria* occurs in the blood of North American frogs, in a new host, *Rana sylvatica*, and in a new locality, near Quebec City, Canada. Until the development into the adult has been worked out experimentally, it cannot be accurately named, but, for convenience in description, it is provisionally named *Microfilaria ranae-sylvaticae* n.sp., with characters as herein set forth.

The effects of some of these Haematozoa on their hosts

The Haematozoa herein described have rarely shown any macroscopical pathological effects on their hosts. Microscopically, infected erythrocytes are altered, as stated in preceding sections. Some differences of the differential leucocyte counts of infected and normal frogs have been found, comparison having been made between hosts of the same sex and age, free from worm infections. Samples only of such counts can be given.

Rana catesbiana, *full-grown male, infected with* Trypanosoma rotatorium

	Normal	Infected
Polymorphonuclears	69	62
Mononuclears	6	9
Lymphocytes	22	27
Eosinophiles	3	2

Rana pipiens, *full-grown male, infected with haemogregarines*

	Normal	Infected
Polymorphonuclears	72	64
Mononuclears	6	10
Lymphocytes	18	22
Eosinophiles	4	4

Bufo americanus, *infected with* Trypanosoma montrealis

	Normal mature male and female	Infected mature male	Infected mature female
Polymorphonuclears	70	68	69
Mononuclears	8	8	6
Lymphocytes	21	22	23
Eosinophiles	1	2	2

Infections of adult, mature frogs with trypanosomes and haemogregarines seem to produce slight decreases in numbers of polymorphonuclear leucocytes, with corresponding increases in mononuclear leucocytes. Infected toads may show the same variation or, as in the case cited, the decrease in polymorphonuclears in both sexes is accompanied by an increase in lymphocytes.

In young frogs and toads, where the blood picture is not yet stabilized, the results of blood counts are very different, and it is doubtful whether reliable conclusions can be made from them. Thus, a normal young male *Rana catesbiana* had 60% polymorphonuclear, 20% mononuclear, 18% lymphocytes, 2% eosinophile leucocytes. A young male of the same age infected with *Trypanosoma rotatorium* had 54% polymorphonuclear, 25% mononuclear, 19% lymphocytes and 2% eosinophile leucocytes. Similarly, a normal young male *Rana pipiens* had 64% polymorphonuclear, 14% mononuclear, 18% lymphocytes and 4% eosinophile leucocytes, while a male of the same age, infected with haemogregarines had 58% polymorphonuclear, 22% mononuclear, 16% lymphocytes and 4% eosinophile leucocytes.

There are marked differences in the differential leucocyte counts of normal young and adult frogs. In infected ones the same trend is observed as in adults. It has been difficult to compare blood counts of female frogs, as in most cases they harboured trematodes or nematodes, the presence of which alters the blood picture. Laboratory-bred frogs and toads were not considered suitable for normal counts, as frequently they refused to feed sufficiently and did not live under natural conditions.

The spleens of twenty ranids harbouring trypanosomes were measured and compared with those of uninfected frogs. There was a slight increase in size, and the spleen pulp was paler and more friable in the infected hosts.

In general, the newer the parasite is to its host, the greater is its effect on that host. Tolerance becomes established with long habituation. Young hosts, being more plastic, show greater variability in blood relations to Haematozoa. They also are more easily infected experimentally.

SUMMARY

Some new species of Mastigophora and Sporozoa and a *Microfilaria*, parasitic in fish, frogs and toads in eastern Canada, are described and illustrated.

Trypanosoma percae n.var. *canadensis* from the yellow perch, *Perca flavescens*, is monomorphic, smaller, with a shorter, broader body and shorter free flagellum than *Trypanosoma percae* from European *Perca fluviatilis*. *Trypanosoma myoxocephali* n.sp. from the longhorn sculpin, *Myoxocephalus octodecimspinosus*, is small, with a narrow undulating membrane. It is the first trypanosome to be recorded from *Myoxocephalus*. *Trypanosoma lavalia* n.sp. from *Bufo americanus* is delicate, with a prominent undulating membrane with two myonemes. *Trypanosoma gaumontis* n.sp. from *Bufo americanus* is

small, crook-like, with undulating membrane with deep folds and one myoneme very near the bordering flagellum. There is no free flagellum. *Trypanosoma montrealis* n.sp. from *Bufo americanus* is long and sinuous, with very short free flagellum and without chromatoid granules in the cytoplasm.

Trypanosoma rotatorium (Mayer) is described from *Rana catesbiana* from eight sites, *R. clamitans* from three sites, and *R. pipiens* from three sites in Quebec Province. *Trypanosoma inopinatum* Ed. & Et. Sergent is recorded from *Rana catesbiana* and *R. pipiens*. A form similar in dimensions and morphology except for a much shorter free flagellum has been found in *R. catesbiana* from St Eustache.

Spirochaeta manitoui n.sp. is described from the blood of *Rana catesbiana* from Lake Manitou.

Lankesterella canadensis n.sp. from *Rana catesbiana* from Montreal and Lake Manitou is described and illustrated. Intracorpuscular forms have polar vacuoles, schizogony occurs in endothelial cells of the blood vessels of the liver and kidney, and multinucleate oocysts have been observed.

Haemogregarina myoxocephali n.sp. from the longhorn sculpin, *Myoxocephalus octodecimspinosus*, and *Haemogregarina urophysis* n.sp. from the white hake, *Urophysis tenuis*, are described. Two other haemogregarines sens lat. from species of *Rana* and *Bufo americanus* are described. *Haemogregarina aeglifini* Henry is recorded from a new host, *Gadus callarias* from off Labrador, *Haemogregarina platessae* Lebailly from *Pseudopleuronectes americanus* and *Haemogregarina bigemina* Laveran & Mesnil from the eel pout, *Zoarces angularis*, the hosts being new.

Plasmodium catesbiana n.sp. from *Rana catesbiana* from the Back River, Montreal, is a small species, with 'signet ring' and amoeboid trophozoites, schizonts with up to eight merozoites clustered round coarse pigment granules and gametocytes showing slight sexual dimorphism. *Plasmodium bufonis* n.sp. occurs in *Bufo americanus* from Laval des Rapides. The schizonts are large, oval, and give rise to eight merozoites arranged en barillet, the pigment usually being at one pole. Possibly micro- and macromerozoites may be produced. Macrogametocytes are oval and deep-staining; microgametocytes are vermicular. Both have pigment.

A haemosporidian, different from but probably allied to *Haemogregarina bigemina*, is described from the black sea bass, *Centropristis striatus*.

Haemoproteus laurentiae n.sp. occurs in *Bufo americanus* from the banks of the St Lawrence at Montreal South and at Lake Manitou. Its pigment is characteristic, consisting of rod-like or bacilliform distributed granules. *Haemoproteus lavalia* n.sp. from *Bufo americanus*, from Laval des Rapides, has pigment restricted to curved bands of rounded to somewhat angular granules. Uninucleate parasites have been observed in extremely few endothelial cells of the lungs. *Haemoproteus lanoraiea* n.sp. from *Bufo americanus*, from Lanoraie, has very pale yellow to light brown pigment granules, densely crowded at one pole and forming a compact granular cap. Oval parasites have been found in endothelial cells of the lungs.

Leucocytozoon salvelini n.sp., parasitic in the leucocytes of the speckled trout, *Salvelinus fontinalis*, is described.

Dactylosoma salvelini n.sp., parasitic in *Salvelinus fontinalis* from a Laurentian stream, is the second species of *Dactylosoma* to be described and the third to be notified from fish. It has fan- to wedge-shaped schizonts producing eight merozoites. Its gametocytes are

not markedly different from one another in shape but differ in cytoplasmic staining and in nuclear structure. *Dactylosoma sylvatica* n.sp., parasitic in *Rana sylvatica* from near Quebec City, produces eight merozoites and has oval and reniform gametocytes.

The morphology of a *Microfilaria* from *Rana sylvatica* is described.

The effects of the Protozoa on the host cells are described for each organism, and some differential leucocyte counts for comparable normal and parasitized Amphibia are given in the concluding section.

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EXPLANATION OF PLATES II–IV

All figures were drawn using an Abbé-Zeiss camera lucida. On Pls. III and IV the cytoplasm of erythrocytes is not indicated, that of endothelial cells is shown diagrammatically by dots and the nuclei of erythrocytes are in outline only.

PLATE II

Figs. 1, 2. *Trypanosoma percae* n.var. *canadensis*. ×2600.

Fig. 3. *Trypanosoma myoxocephali* n.sp. ×1350.

Fig. 4. *Trypanosoma lavalia* n.sp. ×1350.

Figs. 5, 6. *Trypanosoma gaumontis* n.sp. ×1350. The myoneme on the undulating membrane should be nearer the bordering flagellum.

Figs. 7–10. *Trypanosoma montrealis* n.sp. Figs. 7, 9, 10, ×1100; Fig. 8, ×480.

Figs. 11–18. *Trypanosoma rotatorium* (Mayer). ×1350.

Fig. 11. Trypanosome without free flagellum but with well-developed undulating membrane and myonemes.

Fig. 12. Trypanosome with short free flagellum.

Fig. 13. Trypanosome with relatively long free flagellum.

Fig. 14. Dividing form with two nuclei still connected, two kinetoplasts and partly divided undulating membrane.

Fig. 15. Multiple fission.

Figs. 16–18. Stages in formation of the non-flagellate or latent body.

Fig. 16. Body contracting.

Fig. 17. Body almost rounded, nucleus and kinetoplast in juxtaposition.

Fig. 18. Complete, rounded non-flagellate.

Figs. 19, 20. *Spirochaeta manitoui* n.sp. ×1800. The chromatin bars are too wide in these drawings.

PLATE III

Figs. 1–8. *Lankesterella canadensis* n.sp.

Fig. 1. Intracorpuscular form showing nucleus with karyosome and polar vacuoles. ×1200.

Fig. 2. Intracorpuscular form with vacuoles and nucleus of chromatin granules. ×1350.

Fig. 3. Larger intracorpuscular form. Erythrocyte omitted. ×1500.

Fig. 4. Extracorpuscular vermicule. ×1350.
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PLATE IV

Figs. 1–7. *Leucocytozoon salvelini* n.sp. ×1350.
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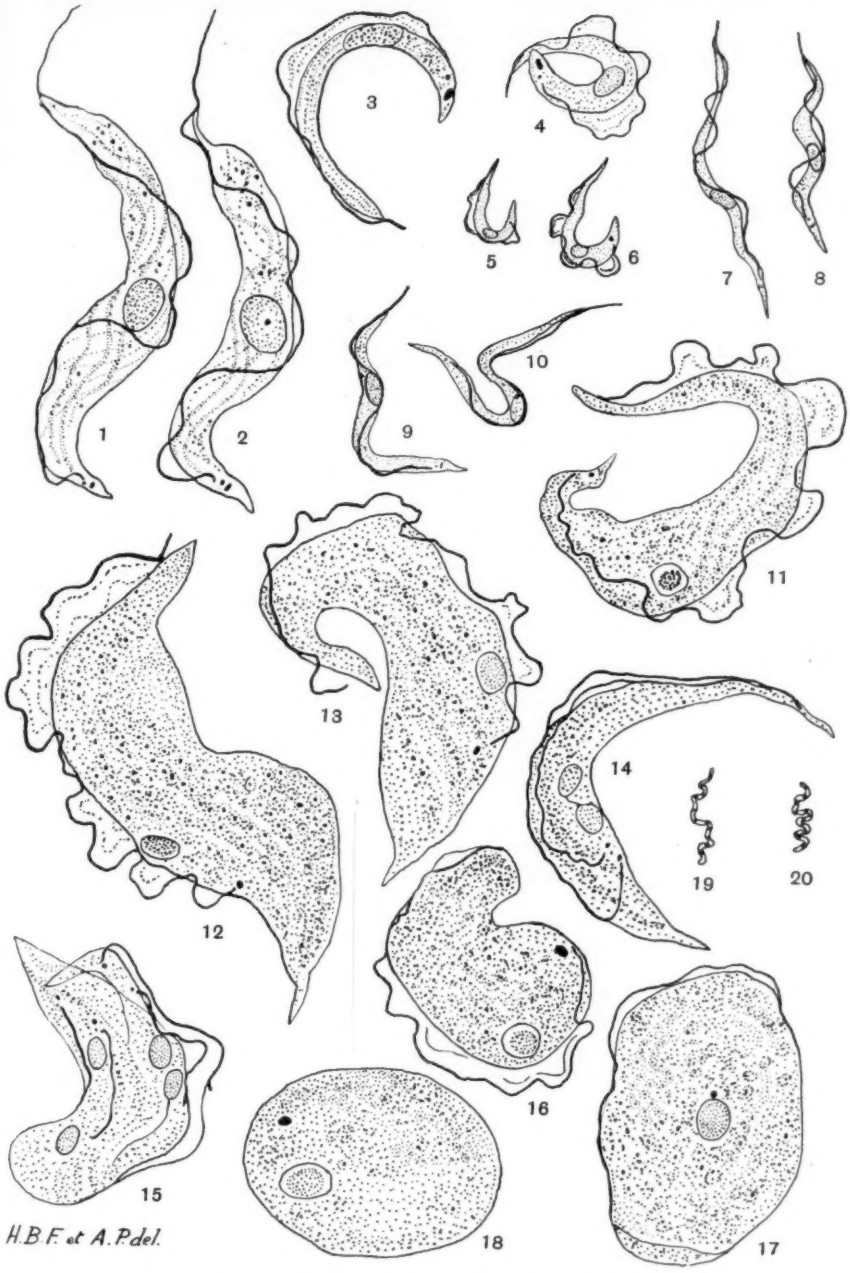

Trypanosomes from Canadian fishes and Amphibia.

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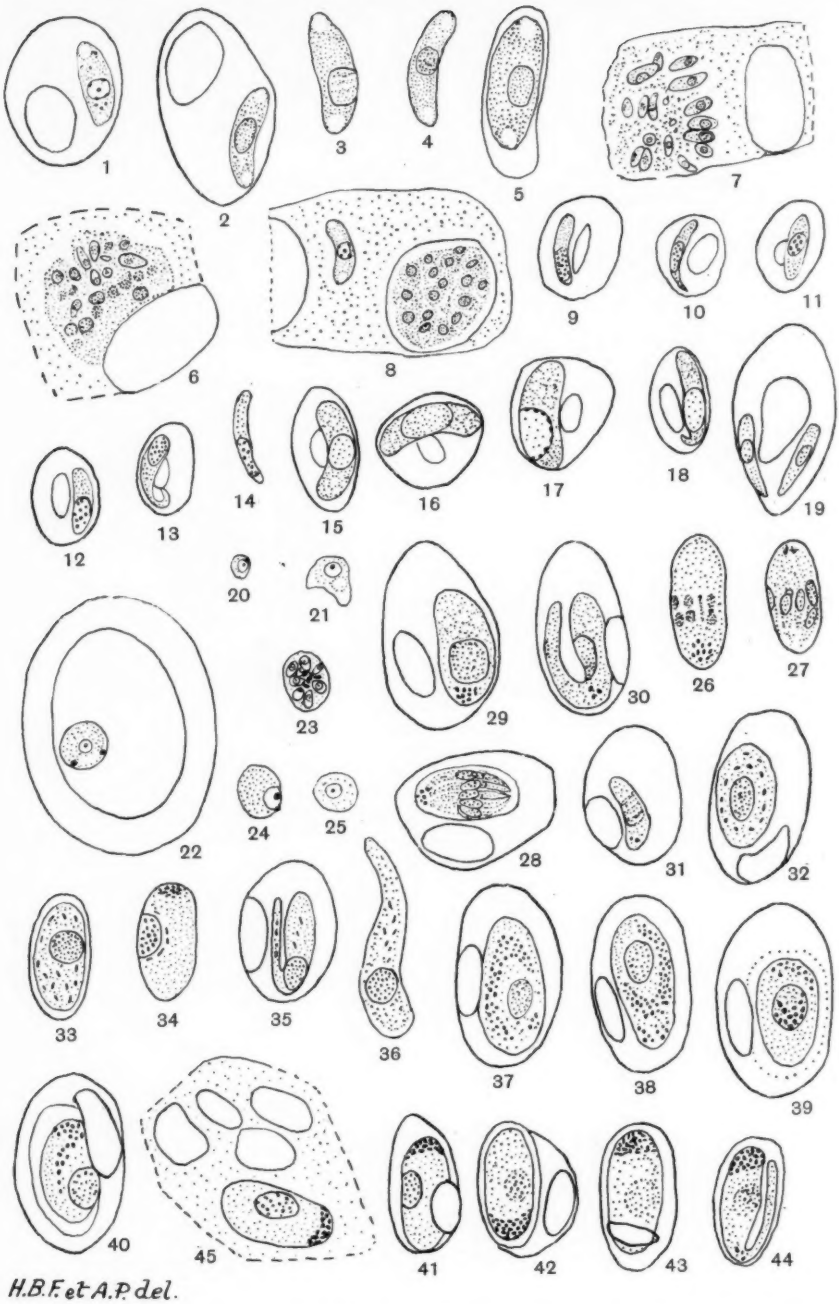

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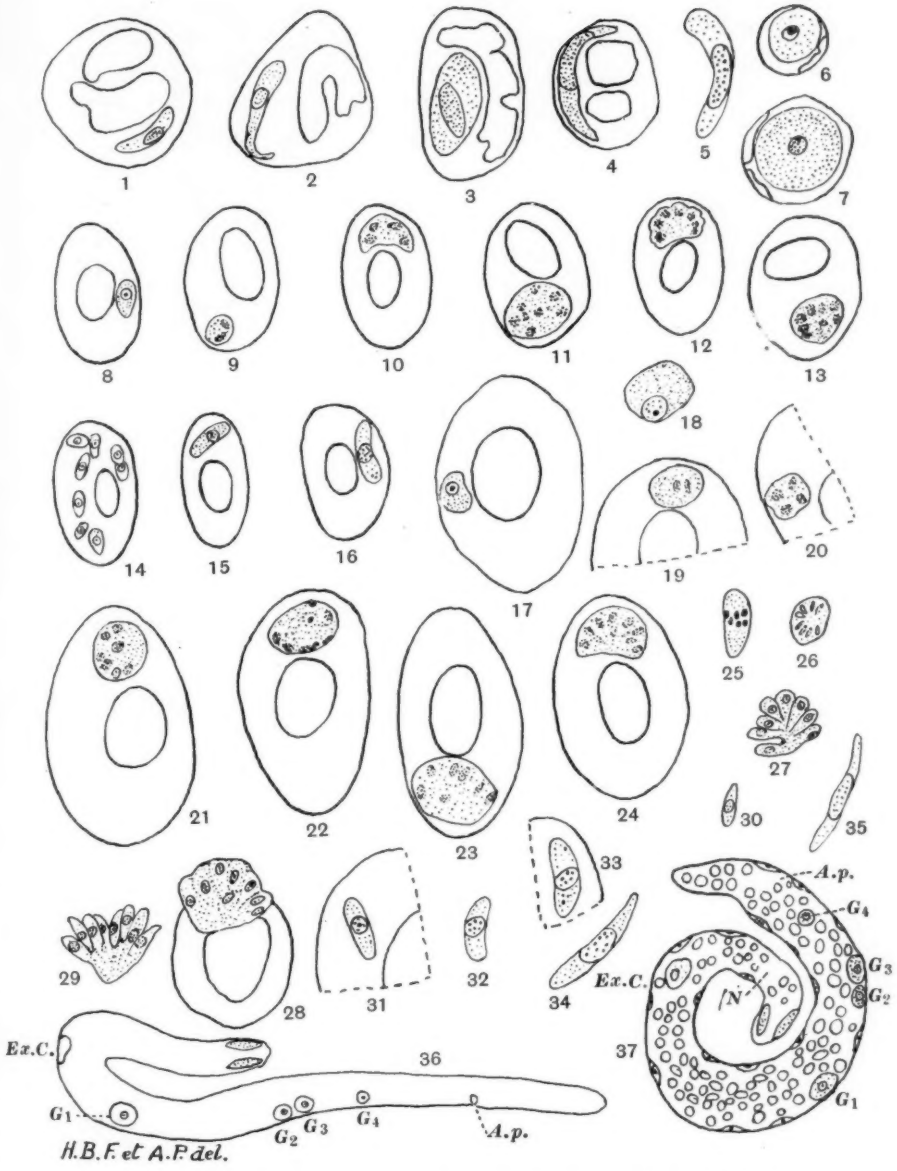
Lankesterella, Haemogregarina, Plasmodium and *Haemoproteus* from Canadian fishes and Amphibia.



Leucocytozoon, *Dactylosoma* and *Microfilaria* from Canadian fishes and Amphibia.

THE INACTIVATION OF PLANT VIRUSES BY RADIATIONS

II. THE RELATION BETWEEN INACTIVATION DOSE AND SIZE OF VIRUS

By D. E. LEA[1] and KENNETH M. SMITH, F.R.S., *Strangeways Research Laboratory, and Plant Virus Research Station, Cambridge*

(With 3 Figures in the Text)

1. Introduction

In previous papers (Lea & Smith, 1940, referred to as Paper I; Lea, 1940*a*) we have suggested that there is a connexion between the dose of X-rays or of a radioactive radiation required to inactivate a plant virus and the size of the particles of the virus, and that a determination of the inactivation dose may in fact be used to give an estimate of the size of the virus. Recently, Luria & Exner (1941) have described experiments on the inactivation of bacteriophage by X-rays, and have calculated estimates of the sizes of the phages by this method. In the present paper we give data of the inactivation of a number of plant viruses by X-rays, and by the alpha- and gamma-rays of radium.

Radiations of the sort here considered dissipate their energy in the material irradiated by ionization, that is, the ejection of electrons from individual atoms. The significance of ionization from the present point of view is simply that *when an atom is ionized the molecule of which it is a part almost certainly suffers chemical change.* In the references cited we have given evidence, which we shall not here recapitulate, that when a plant-virus particle is inactivated, a single ionization suffices. In view of the crystallizability of several of the plant viruses it is plausible to regard the smallest units exhibiting the properties of the virus as molecules. Thus the result that a single ionization is capable of inactivating a virus particle is not surprising. The method of determining the size of the virus from the radiation experiments depends on the presumption that the ionization of any atom in the molecule leads to the inactivation of the virus. The possibility cannot be excluded, however, that ionization of some of the atoms of the molecule may lead to local changes without actually causing inactivation. Some of these changes may result in a modification of the symptoms of the plant disease caused by the virus without destroying its fundamental property of multiplying in the cells of the host. One would then have *mutation* of the virus caused by the radiation, such as has been claimed by Gowen (1939) and by Kausche & Stubbe (1939). Such a change is likely to be less probable than inactivation, a presumption which can be tested as soon as quantitative data are available for the induction of mutations by irradiation of viruses. In this event, atoms, the ionization of which leads to viable mutations rather than inactivation, will constitute only a small part of the whole molecule.

On the other hand, it is probable that the viruses form loose associations with water, so that in aqueous suspension the virus is hydrated, while when dried the water is lost without permanent loss of infectivity. The ionization of such unessential water molecules is not therefore likely to inactivate the virus particle. Hence we conclude that the size

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of the virus deduced from the radiation experiments (conveniently referred to as the 'target' size) will be the unhydrated size, and may further be somewhat underestimated if structural change can occur without loss of infectivity.

Briefly, the radiation method measures the size of that part of the virus which is essential for its power to multiply in the host plant and to produce lesions in it. In larger entities (such as vaccinia virus, Lea & Salaman, 1942; bacteria, Lea, Haines & Bretscher, 1941), where there is a differentiation between the genes carrying the heredity and the genetically inert material present in much greater bulk, the 'target' size inferred from the radiation experiments is very much less (less than 1%) than the total size of the organism. The use of the radiation method for the determination of the sizes of plant viruses is tantamount to the presumption that no such differentiation here exists. Alternatively, the argument may be reversed and the approximate agreement that is obtained between the 'target' size and the size of the virus determined by other means taken as proof that no such differentiation exists in the plant viruses, thus drawing a fundamental distinction between the smaller viruses and the larger viruses such as vaccinia.

2. Inactivation by gamma-rays

Gamma-rays are the simplest radiations to use in these experiments, both from the point of view of the technical ease of making the irradiation and the simplicity of the calculation of size of virus from inactivation dose. In these experiments the irradiation lasted some days, and took place in the refrigerator.

Five small cylinders were available each containing 200 mg. of radium, and these were arranged symmetrically round the perimeter of a circle of radius 1 cm., at the centre of which was the specimen to be irradiated. This arrangement gave a satisfactorily uniform radiation field. The filtration used was sufficient to ensure that only gamma-rays reached the specimen. The intensity was measured by means of a small graphite ionization chamber and was found to be 7700 r./hr. The roentgen is a unit of dose based on the electrical measurement of the ionization produced by the radiation in air. For our purposes we require the inactivation dose in terms of ionization in the material irradiated. This is proportional to the dose in roentgens, 1 r. of gamma-rays corresponding approximately to 1·74 ionizations per 10^{-12} g. in a material composed mainly of the lighter chemical elements.

The virus preparations in some instances were liquid suspensions (either clarified sap or suspensions which had been purified in varying degrees) and in others, films dried on to glass. It is important to show that the inactivation rates are equal in the two states. A rate of inactivation greater in water than in the dry state would indicate that an indirect effect was occurring, energy absorbed by the water being transferred to the virus, which could thus be inactivated without itself being directly ionized by the radiation. Enzymes (Dale, 1940) irradiated in aqueous solution are inactivated by this indirect action via the water, and under some circumstances the indirect effect contributes to the inactivation of bacteriophages (Luria & Exner, 1941).

In Fig. 1 are shown the inactivation curves of several viruses irradiated by gamma-rays. It is seen that the same rates of inactivation were obtained dry and wet in those instances in which both experiments were made, indicating that in aqueous suspension there is no appreciable indirect effect via the water. Fig. 1 of Paper I demonstrated the same result for X-rays. Some of the viruses could only be irradiated wet on account of

the loss of activity which occurred on drying. In Fig. 1 we plot as ordinate the natural logarithm (to base $e=2{\cdot}718$) of the ratio of the lesion counts obtained with exposed and control virus samples, which were inoculated by the half-leaf method as in Paper I. The linearity (apart from random experimental variations) of the curves so obtained indicates a constant rate of disappearance of infective virus during the treatment, as in a monomolecular reaction, and is one of the arguments (see Paper I) for the effect being ascribed to a single ionization process and not to the cumulative effect of separate processes.

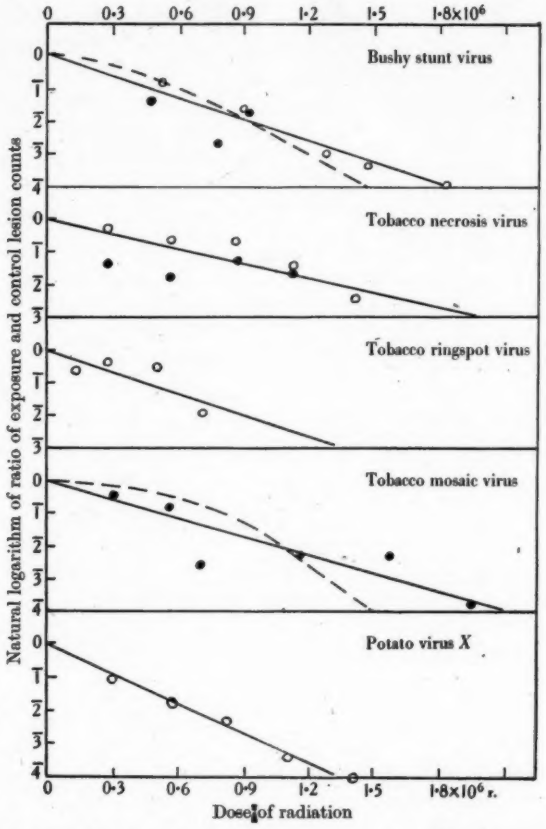

Fig. 1. The inactivation of various viruses by gamma-rays. ○ irradiated wet. ● irradiated dry.

The inactivation dose, defined as the dose required to reduce the number of infective virus particles to a fraction $e^{-1}=0{\cdot}37$ of the control number is read off from the graphs and tabulated in Table 1 for each virus. In the case of tobacco mosaic virus the inactivation dose shown in the table is the mean of four determinations made on different preparations, ranging from 0·26 to $0{\cdot}53\times10^6$ r., while the graph in Fig. 1 refers to one of these determinations, namely, that giving an inactivation dose $0{\cdot}53\times10^6$ r.

With a dose equal to the inactivation dose therefore 37% of the virus particles must have escaped a 'hit', where by a 'hit' we mean the production of ionization in the virus,

or more exactly, in its 'target' volume, and it follows that on the average there has been one hit per virus particle. This deduction is not based on any special property of viruses or of radiation, but is a purely statistical result. For example, if in a haemocytometer there is an average of one cell per square, we shall expect to find about 37 % of squares empty.

Thus the inactivation dose is that dose which produces an average of one hit per virus particle. If the ionizations were produced by the radiation singly, this would mean an average of one ionization per virus particle. Actually there is a tendency for the ionization to occur in small clusters sufficiently compact for the cluster rather than the single ionization to act as the unit. Since the average size of a cluster is about three ionizations, the inactivation dose D produces an average of three ionizations per virus particle. But a dose D causes the production of 1·74 D ionizations per 10^{-12} g., i.e. of

Table 1. *Gamma-ray inactivation doses and inferred virus sizes*

Virus ...	Bushy stunt	Tobacco necrosis	Tobacco ringspot	Tobacco mosaic	Potato virus *X*
Inactivation dose (r.)	$0{\cdot}45 \times 10^6$	$0{\cdot}67 \times 10^6$	$0{\cdot}46 \times 10^6$	$0{\cdot}37 \times 10^6$	$0{\cdot}33 \times 10^6$
Molecular weight	$2{\cdot}32 \times 10^6$	$1{\cdot}57 \times 10^6$	$2{\cdot}27 \times 10^6$	$2{\cdot}80 \times 10^6$	$3{\cdot}14 \times 10^6$
Diameter (mμ)	17·4	15·3	17·2	—	—

Table 2. *Virus sizes by other methods*

(General reference, Markham, Smith & Lea, 1942)

Virus	Diameter mμ	Mol. wt.	Method	Reference
Bushy stunt	13–20	$1\text{–}3{\cdot}5 \times 10^6$	Filtration	Smith & MacClement (1941)
	29·2	10·6	X-ray diffraction	Bernal, Fankuchen & Riley (1938)
	~26	~7·8	Electron microscope	Stanley & Anderson (1941)
Tobacco necrosis	13–20	1–3·5	Filtration	Smith & MacClement (1941)
	25	7·2	Sedimentation, $s_{20} = 112$	Price & Wyckoff (1939) recalculated
	~20	~3·5	Electron microscope	Stanley & Anderson (1941)
Tobacco ringspot	13–20	1–3·5	Filtration	Smith & MacClement (1941)
	19	3·4	Sedimentation, $s_{20} = 115$, $V = 0{\cdot}636$	Stanley (1939)
Tobacco mosaic	$15{\cdot}2 \times 280$	41·9	X-ray diffraction and electron microscope	Bernal & Fankuchen (1941) Stanley & Anderson (1941)
Potato virus *X*	$9{\cdot}8 \times 433$	26	Sedimentation, $s_{20} = 113$ and viscosity	Loring (1938)

three ionizations per $3 \times 10^{-12}/1{\cdot}74\ D$ g. of the irradiated virus. Thus the weight of the virus must be $3 \times 10^{-12}/1{\cdot}74\ D$ g. Multiplying by $6{\cdot}023 \times 10^{23}$ to obtain the molecular weight from the weight in grams, we obtain the formula

$$\text{Molecular weight} = \frac{1{\cdot}038 \times 10^{12}}{\text{Inactivation dose in roentgen}}$$

for the molecular weight of the virus particle or, strictly, of the target.

If the diameter of the virus is required, we deduce it from the formula

$$\tfrac{4}{3}\pi r^3 \rho = 3 \times 10^{-12}/1{\cdot}74\ D,$$

whence $2r = 1330/D^{\frac{1}{3}}$ mμ, ρ being the density, about 1·4 for plant viruses.

In this fashion we calculate the molecular weights and diameters listed in Table 1. Potato *X* and tobacco mosaic viruses are known to be rod-shaped, and hence for these viruses we calculate only the molecular weight. In Table 2 for comparison we give the estimates of size given by other methods, and it is evident that the diameters of the

spherical viruses by the radiation method lie within the range of sizes given by other methods, though probably somewhat smaller than the most reliable of the other methods (see review by Markham, Smith & Lea, 1942). In Table 3 we tabulate the ratio of the target molecular weight to what we consider to be the best estimate available of the particle weight. The ratio is from 0·22 to 0·67 for the three spherical viruses, which corresponds to a ratio of diameters of 0·6–0·9.

Table 3. *Ratio of target size to particle size*

Virus	Mol. wt. Target	Mol. wt. Particle	Ratio	
Bushy stunt	$2{\cdot}32 \times 10^6$	$10{\cdot}6 \times 10^6$	0·22	Spherical viruses
Tobacco necrosis	1·57	7·2	0·22	
Tobacco ringspot	2·27	3·4	0·67	
Tobacco mosaic	2·80	41·9	0·07	Rod-shaped viruses
Potato *X*	3·14	26	0·12	

3. Inactivation by X-rays and alpha-rays

In addition to the experiments with gamma-rays, experiments were made in some instances with two wave-lengths of soft X-rays (1·5 and 8·3 A.), and with alpha-rays. The low penetrating power of these radiations necessitated the use of the virus dried in thin films, and experiments were necessarily confined to those viruses which retained their infectivity after this treatment. Making up the virus suspension in ½% lactose solution before drying was found to be advantageous in some cases. Single drops of the virus suspension were placed on cleaned cover-slips and allowed to dry. The drops dried with a thick rim, and this was scraped away with a razor blade leaving an apparently uniformly thin film of the virus which was trimmed to a size of 5 mm. square. The superficial mass of the film was determined by weighing, and was usually about 0·5 mg./cm.^{2} The X-rays of wave-length 8·3 A. are appreciably absorbed even by this small thickness, but a correction can be made since their absorption coefficient is known (1·2 mg.$^{-1}$ cm.^{2}). No correction is needed in the case of the other radiations.

The X-rays were produced by a laboratory-built, continuously evacuated, X-ray tube. This tube and the method of measuring the radiations from it have been described (Lea, 1941). The dose rate used was about 10^4 r./min. in the case of the 1·5 A. radiation, and 10^5 r./min. in the case of the 8·3 A. radiation.

The alpha-rays were obtained from a source of polonium of the order of a tenth of a millicurie, deposited on a silver disk and held about 2 mm. from the virus film. The intensity was measured by means of a shallow ionization chamber and was usually about 10^5 r./hr. Irradiations sometimes lasted for more than 24 hr., and were carried out in the refrigerator.

The extra complications of technique involved in the use of the viruses in thin films and the variable loss of activity which occurred in the drying made it impossible to obtain high precision in these experiments. Fortunately, the differences in slope between the inactivation curves of the different radiations are considerable, and show up unmistakably despite the large fluctuations of the experimental points (Fig. 2).

We mentioned earlier that the calculation of the estimates of size from experiments with X-rays and alpha-rays is a little more complicated than with gamma-rays. This is due to the fact that the clusters of ionization are not distributed independently, but lie

on the paths of ionizing particles which traverse the material during the irradiation. With gamma-rays the clusters are separated by distances so great that two or more clusters very rarely fall in the same virus particle, but with X-rays or alpha-rays they lie closer together, and a 'hit' usually involves a number F of clusters instead of only one. The inactivation dose is thus increased by the factor F. F can be calculated as a function of ξ, the ratio between the diameter $2r$ of the target and the mean separation L of consecutive clusters along the path of the ionizing particle. $F=1$ for ξ much less

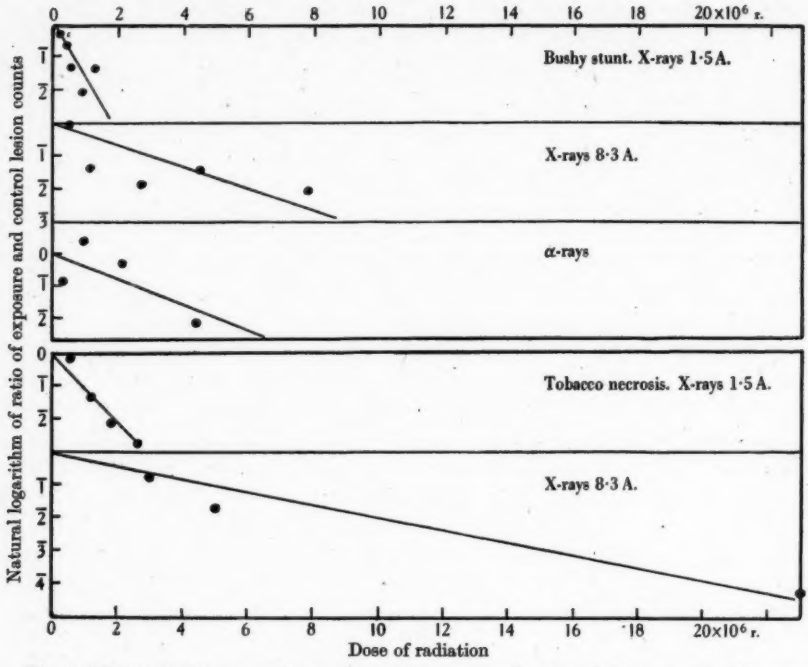
Bushy stunt. X-rays 1·5 A.
X-rays 8·3 A.
α-rays
Tobacco necrosis. X-rays 1·5 A.
X-rays 8·3 A.
Natural logarithm of ratio of exposure and control lesion counts
Dose of radiation
0 2 4 6 8 10 12 14 16 18 20×10⁶ r.

Fig. 2. The inactivation of bushy stunt and tobacco necrosis viruses by X-rays and alpha-rays.

Table 4. *Physical data of radiations*

Radiation	Gamma-rays	X-rays wave-length (A.) 0·15	1·5	8·3	Alpha-rays
$1/L$, in matter of density 1·4 (clusters per μ)	7	29·4	70	336	1820
1 r. = in matter of density 1·4 (ionization per μ^3)	2·44	2·38	1·82	2·13	3·68

than 1, and $F=\frac{2}{3}\xi$ for ξ much greater than 1. For intermediate values of ξ the values of F may be read off from a graph given in another publication (Lea, 1940*b*). The values of $1/L$ for the various radiations are given in Table 4.

A set of curves for tobacco necrosis virus and tomato bushy stunt virus using several radiations are given in Fig. 2, and the inactivation doses are tabulated in Tables 5 and 6, while Table 7 gives similar data for tobacco mosaic virus.

It is seen that the inactivation doses on the whole increase from left to right in the tables, as is to be expected on this theory, according to which the inactivation doses should be greater for radiations producing ionizations close together.

To facilitate the deduction of virus size from inactivation dose we have computed the curves shown in Fig. 3, from which may be read off the target size corresponding to any given inactivation dose. To show how these curves are computed, we carry through

Table 5. *Tomato bushy stunt virus*

Radiation	Gamma-rays	X-radiation 1·5 A.	X-radiation 8·3 A.	Alpha-rays
Inactivation dose (r.)	$0{\cdot}45 \times 10^6$	$0{\cdot}62 \times 10^6$	$3{\cdot}10 \times 10^6$	$2{\cdot}56 \times 10^6$
Inactivation dose (ionizations per μ^3)	$1{\cdot}10 \times 10^6$	$1{\cdot}13 \times 10^6$	$6{\cdot}60 \times 10^6$	$9{\cdot}41 \times 10^6$
Inferred virus diameter (mμ)	17·4	20·1	14·3	27·0

Table 6. *Tobacco necrosis virus*

Inactivation dose (r.)	$0{\cdot}67 \times 10^6$	$0{\cdot}94 \times 10^6$	$5{\cdot}15 \times 10^6$	—
Inactivation dose (ionizations per μ^3)	$1{\cdot}63 \times 10^6$	$1{\cdot}71 \times 10^6$	$10{\cdot}9 \times 10^6$	—
Inferred virus diameter (mμ)	15·3	17·1	11·5	—

Table 7. *Tobacco mosaic virus*

Inactivation dose (r.)	$0{\cdot}37 \times 10^6$	$0{\cdot}43 \times 10^6$	$1{\cdot}49 \times 10^6$	$1{\cdot}90 \times 10^6$
Inactivation dose (ionizations per μ^3)	$0{\cdot}90 \times 10^6$	$0{\cdot}78 \times 10^6$	$3{\cdot}17 \times 10^6$	$6{\cdot}95 \times 10^6$

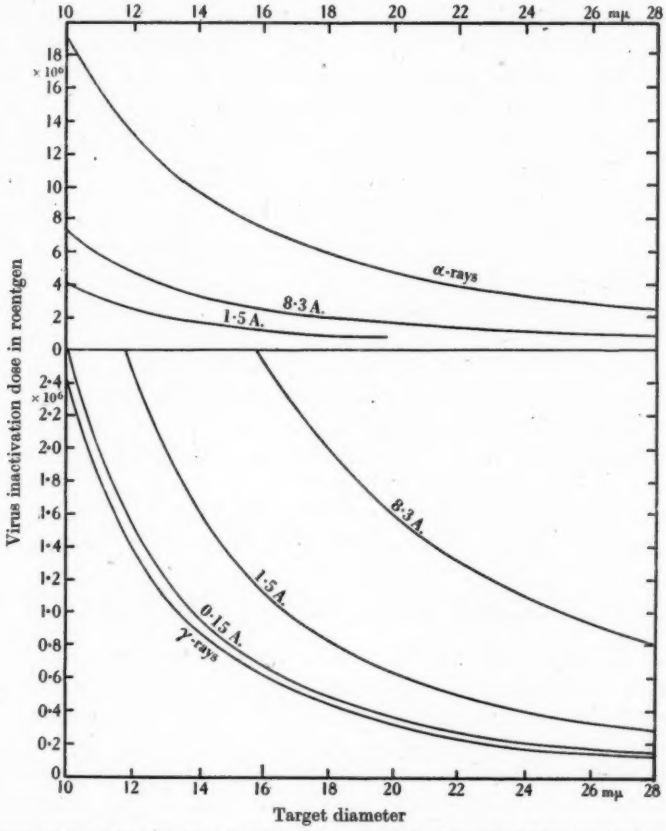

Fig. 3. Curves relating inactivation dose and target diameter. Computed for virus density 1·4 and the following radiations: alpha-rays; X-rays of wave-length 8·3, 1·5 and 0·15 A.; gamma-rays.

the calculation of the inactivation dose of X-rays wave-length 1·5 A. to be anticipated for a target diameter $2r = 20$ m$\mu = 0{\cdot}020\,\mu$.

X-rays of this wave-length produce $1/L = 70$ ionization clusters per micron track in virus material density 1·4 (Table 4). Therefore $\xi = 2r/L = 0{\cdot}02 \times 70 = 1{\cdot}40$. Referring to the curve of F against ξ given in Lea (1940*b*), we read $F = 1{\cdot}60$. Thus the average number of ionizations in the target when the virus is inactivated is 1·60 clusters = 4·80 ionizations. The volume of the target being $\frac{1}{6}\pi\,(0{\cdot}02)^3 = 4{\cdot}189 \times 10^{-6}\,\mu^3$, the inactivation dose is 4·80 ionizations per volume of $4{\cdot}189 \times 10^{-6}\,\mu^3$, i.e. $1{\cdot}146 \times 10^6$ ionizations per $\mu^3 = 1{\cdot}146 \times 10^6/1{\cdot}82 = 0{\cdot}63 \times 10^6$ r. (see Table 4 for the factor 1·82).

The estimates of the size of a given virus deduced from experiments with different radiations agree tolerably well. The fact that the estimates show no systematic increase or decrease from left to right in Tables 5 and 6 shows that the trend of the experimental inactivation doses, which does show a systematic increase from left to right, is fairly satisfactorily explained by the present method of calculation.

It is not claimed that this method of calculation is exact, since the spatial distribution of ionization is rather more complicated than has been allowed for here. The principal inadequacy of the calculation is probably in the neglect of the delta-rays produced by the alpha-rays. Neglect of the delta-rays will lead to the estimates of size deduced from the alpha-ray experiments by use of Fig. 3 being rather too large.

No estimates of size were made for tobacco mosaic virus since this is known to be non-spherical. Gowen (1940) has also obtained with this virus a variation of inactivation dose with wave-length of X-rays in the same direction as the present results and even more marked.

High accuracy is not claimed for the experimental figures in Tables 5, 6 and 7. When repeating the experiments we have not obtained good reproducibility, which may be due to the experimental difficulties involved in the use of thin films of virus, or may be a real difference between different batches of virus. Since, however, in every experiment with each of the three viruses we have always obtained inactivation doses increasing in the order gamma-rays, X-rays, alpha-rays, we consider this qualitative result to be established with certainty, though the numerical values listed in the tables are only approximate. The considerable difference between the inactivation dose of tobacco necrosis virus reported in Paper I ($2{\cdot}8 \times 10^6$ r.) and that now given based on more recent experiments ($0{\cdot}94 \times 10^6$ r.) we are inclined to ascribe to the known polydispersity and variability of this virus, as shown by sedimentation (Pirie, Smith, Spooner & MacClement, 1938), filtration (Smith & MacClement, 1940), and serological behaviour (Bawden, 1941).

4. The relation between target size and virus size

Comparison of Tables 1 and 2 shows that the target sizes found for the three nearly spherical viruses, namely, bushy stunt, tobacco necrosis, and tobacco ringspot, are within the range of the sizes deduced by other methods. If, however, we accept the assessment of Markham *et al.* (1942), who consider the larger estimates of size to be more accurate than the smaller estimates (which are based for the most part on filtration data), we conclude that the target size is somewhat smaller than the size of the whole virus. Examination of the premises of the radiation method, set out in the Introduction, make it clear that the explanation is to be looked for in one or more of the following possibilities:

(1) The premise that every ionization in a virus produces change in its molecular structure may not be valid. The reason for making this premise is partly that the energy given to an atom which is ionized is much greater than the minimum energy necessary to dissociate the chemical bonds linking the atom in the molecule, and partly that researches (Lind, 1928) on the effects of radiations in inducing chemical change in inorganic materials suggest that this is the primary effect, though the primary effect in many inorganic reactions is followed by a chain reaction increasing the yield, or by recombination reducing the yield of molecules changed per ionization. We consider this explanation unlikely, more especially as the target size is less than virus size even in the case of radiations producing many ionizations in the virus, and not only one or two.

(2) It is possible that ionization of certain atoms in the virus particle may produce changes in molecular structure which still leave the virus capable of multiplying in the host plant and of producing local lesions. The molecular weight of the 'target' would then be less than that of the whole virus, since from the former would be excluded the weight of those atoms which could be ionized without inactivating the virus. If the suggestion that mutations can be produced by irradiation of viruses is confirmed (Gowen, 1939; Kausche & Stubbe, 1939) and a target molecular weight is deduced for mutation, this will need to be added to the target molecular weight for inactivation. There still remains the possibility that some change in molecular structure may be possible without change in the symptoms. Miller & Stanley (1941) have shown, for example, that 70% of the amino groups in tobacco mosaic virus may be acetylated without change in its biological properties.

(3) In the case of viruses which are not crystallizable, and which therefore we are not necessarily justified in regarding as macromolecules, there may be differentiation into genetic and non-genetic parts, corresponding to the differentiation into genes and cytoplasm in higher cells. The target volume relates only to the genetic material, since a single molecule of non-genetic material suffering chemical change cannot plausibly be imagined to lead to inactivation. Such differentiation into genetic and non-genetic material is the explanation advanced for the very much greater difference between target size and particle size in the case of large viruses. Thus the ratio of target weight to particle weight is 1 : 200 in the case of vaccinia. The existence of such large differences in target and particle size has been suggested (Lea & Salaman, 1942) as a means of distinguishing between viruses which are macromolecules and those which are genetically more akin to single-celled organisms. No such large differences occur in any of the plant viruses studied, and since there is no suggestion of the difference between target size and particle size being greater for those plant viruses which have not yet been crystallized than for bushy stunt and tobacco mosaic which have, we regard all the viruses here studied as macromolecules.

(4) The unit commonly regarded as a single virus particle may not be the molecule in the sense of the smallest unit having the properties of the virus. Some viruses, particularly tobacco mosaic, exist in a variety of sizes, presumably aggregates of some minimum unit which would be regarded as the molecule. There is no guarantee that the viruses which appear to be monodisperse in solution exist there in single molecules. The absence of certain expected reflexions in the X-ray diffraction pattern of bushy stunt virus (Bernal *et al.* 1938) may be due to the particle not being the molecule.

If the virus particle in solution, for example, consists of n molecules each of which

by itself is capable of causing an infection, then to make the whole particle non-infective it will be necessary to produce ionization in each of the molecules. Suppose D_0 is the inactivation dose of the single molecule. Then $1-e^{-D/D_0}$ is the probability that dose D will inactivate a given molecule, and $(1-e^{-D/D_0})^n$ the probability that it will inactivate all n, and so inactivate the whole particle. The proportion of virus particles still active after dose D is thus $1-(1-e^{-D/D_0})^n$, in place of e^{-D/D_0} if the particle were a single molecule. In Fig. 1 (bushy stunt) the dotted line has been calculated in this way, supposing that the bushy stunt virus particle, which has been shown by other methods to have a molecular weight $10{\cdot}6\times10^6$, comprises three molecules each of molecular weight $3{\cdot}53\times10^6$, and that the particle remains infective until all three have been separately ionized. While the dotted curve does not fit the experimental points so well as the straight line, the precision we have been able to obtain in these experiments is not sufficient to decide against it. Similarly, the dotted curve in the tobacco mosaic diagram (Fig. 1) is the curve to be anticipated if the virus particle of $15{\cdot}2\times280$ mμ is composed of ten molecules each of molecular weight $4{\cdot}2\times10^6$.

In view of the exceptionally low ratio of target weight to molecular weight shown by tobacco mosaic virus (Table 3) it is considered likely that the particle of size $15{\cdot}2\times280$ mμ is not the smallest infective unit. As regards the spherical viruses, it is not yet possible to say whether the difference between target size and particle size is due to alternatives (2) or (4) discussed above, but in view of the experiments of Miller & Stanley already referred to, it seems likely that alternative (2) plays a part.

Summary

Experiments are described on the inactivation by gamma-rays, X-rays, and alpha-rays of the viruses of tomato bushy stunt, tobacco necrosis, tobacco ringspot, tobacco mosaic and potato virus *X*. Within the errors of the experiment the inactivation curves appear to be exponential, and the inactivation doses increase in the order gamma-rays, X-rays of wave-length 1·5 A., X-rays of wave-length 8·3 A., and alpha-rays.

A theory is given explaining these results and correlating the inactivation dose with the virus size. Estimates of the sizes of the viruses obtained from the radiation experiments lie within the range of the sizes given by other methods, but are somewhat lower than the most probable sizes. Possible explanations of the discrepancy which are discussed are (*a*) the virus particle is not the molecule, in the sense of the smallest infective unit, or (*b*) certain structural changes in the virus molecule produced by the radiation may still leave it infective. Some of these may perhaps show themselves as mutations.

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